AU9468278

(12) PATENT ABRIDGMENT (11) Document No. AU-B-68278/94
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No 691810

(54) Title
METHODS FOR TREATING MUSCLE DISEASES AND DISORDERS

International Patent Classification(s)
(51)[5] A61K 037/10 A61K 037/36
(51)[6] G01N 033/53 G01N 033/68

(21) Application No. . 68278/94 (22) Application Date 06.05.94

(87) PCT Publication Number : WO94/26298

(30) Priority Data

(31) Number	(32) Date	(33) Country
059022	06.05.93	US UNITED STATES OF AMERICA
209204	08.03.94	US UNITED STATES OF AMERICA

(43) Publication Date : 12.12.94

(44) Publication Date of Accepted Application : 28.05.98

(71) Applicant(s)
CAMBRIDGE NEUROSCIENCE

(72) Inventor(s)
ROBERT SKLAR; MARK MARCHIONNI; DAVID I GWYNNE

(74) Attorney or Agent
DAVIES COLLISON CAVE , 1 Little Collins Street, MELBOURNE VIC 3000

(56) Prior Art Documents
AU 47694/93

(57) For treatment of smooth muscle disease, Becker's dystrophy, cardiac muscle disorder arterial sclerosis, vascular lesion, acetylcholine receptor insufficiency.

Claim

1. A method of treating muscle cells to increase mitogenesis, differentiation and/or survival of said muscle cell in a mammal, said method comprising administering to said mammal a polypeptide encoded by pGGF2HBS11, deposited with the A.T.C.C. November 6, 1992 (A.T.C.C. Deposit No. 75347).

38. A method of treating muscle cells to increase mitogenesis, differentiation and/or survival of said muscle cell in a mammal, said method comprising administering to said mammal a 35 kD polypeptide factor having muscle cell mitogenesis differentiation and/or survival inducing properties and isolated from the rat I-EJ transformed fibroblast cell line to said muscle cells, with a pharmaceutically acceptable carrier.

CORRECTED VERSION*

PCT

pages 86-163, description, replaced by new pages 86-152; p
56-180, claims, renumbered as pages 153-167; pages 1/57-
2/57, 26/57-27/57 and 55/57, drawings, replaced by new pages
1/55-9/55, 18/55, 24/55-25/55 and 53/55; pages 12/57-19/57,
21/57-25/57, 28/57-54/57, 56/57 and 57/57, renumbered as pages
10/55-17/55, 19/55-23/55, 26/55-52/55, 54/55 and 55/55; due to late
transmittal by the receiving Office

69275/

INTERNATIONAL APP RATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/10, 37/36	A1	(11) International Publication Number: WO 94/26298 (43) International Publication Date: 24 November 1994 (24.11.94)

(21) International Application Number: PCT/US94/05083

(22) International Filing Date: 6 May 1994 (06.05.94)

(30) Priority Data:

08/059,022	6 May 1993 (06.05.93)	US
08/209,204	8 March 1994 (08.03.94)	US

(71) Applicant (*for all designated States except US*): CAMBRIDGE NEUROSCIENCE [US/US]; Building 700, One Kendall Square, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): SKLAR, Robert [US/US]; 36 Spiers Road, Newton, MA 02159 (US). MARCHIONNI, Mark [US/US]; 24 Twin Circle Drive, Arlington, MA 02174 (US). GWYNNE, David, I. [GB/US]; 77 Grover Street, Beverly, MA 01915 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).

(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

691810

(54) Title: METHODS FOR TREATING MUSCLE DISEASES AND DISORDERS

(57) Abstract

The invention relates to methods of treating diseases and disorders of the muscle tissues in a vertebrate by the administration of compounds which bind the $p185^{erbB2}$ receptor. These compounds are found to cause increased differentiation and survival of cardiac, skeletal and smooth muscle.

* (Referred to in PCT Gazette No.37/1996, Section II)

CORRECTED VERSION

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [5] : A61K 37/10, 37/36 — A1

(11) International Publication Number: WO 94/26298

(43) International Publication Date: 24 November 1994 (24.11.94)

(21) International Application Number: PCT/US94/05083

(22) International Filing Date: 6 May 1994 (06.05.94)

(30) Priority Data:
08/059,022 6 May 1993 (06.05.93) US
08/209,204 8 March 1994 (08.03.94) US

(71) Applicant (*for all designated States except US*): CAMBRIDGE NEUROSCIENCE [US/US]; Building 700, One Kendall Square, Cambridge, MA 02139 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): SKLAR, Robert [US/US]; 36 Spiers Road, Newton, MA 02159 (US). MARCHIONNI, Mark [US/US]; 24 Twin Circle Drive, Arlington, MA 02174 (US). GWYNNE, David, I. [GB/US]; 77 Grover Street, Beverly, MA 01915 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).

(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS FOR TREATING MUSCLE DISEASES AND DISORDERS

(57) Abstract

The invention relates to methods of treating diseases and disorders of the muscle tissues in a vertebrate by the administration of compounds which bind the $p185^{erbB2}$ receptor. These compounds are found to cause increased differentiation and survival of cardiac, skeletal and smooth muscle.

* (Referred to in PCT Gazette No.37/1996, Section II)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

METHODS FOR TREATING MUSCLE DISEASES AND DISORDERS

Background of the Invention

The invention relates to prophylactic or affirmative treatment of diseases and disorders of the musculature by administering polypeptides found in vertebrate species, which polypeptides are growth, differentiation and survival factors for muscle cells.

Muscle tissue in adult vertebrates will regenerate from reserve myoblasts called satellite cells. Satellite cells are distributed throughout muscle tissue and are mitotically quiescent in the absence of injury or disease. Following muscle injury or during recovery from disease, satellite cells will reenter the cell cycle, proliferate and 1) enter existing muscle fibers or 2) undergo differentiation into multinucleate myotubes which form new muscle fiber. The myoblasts ultimately yield replacement muscle fibers or fuse into existing muscle fibers, thereby increasing fiber girth by the synthesis of contractile apparatus components. This process is illustrated, for example, by the nearly complete regeneration which occurs in mammals following induced muscle fiber degeneration; the muscle progenitor cells proliferate and fuse together regenerating muscle fibers.

Several growth factors which regulate the proliferation and differentiation of adult (and embryonic) myoblasts <u>in vitro</u> have been identified. Fibroblast growth factor (FGF) is mitogenic for muscle cells and is an inhibitor of muscle differentiation. Transforming growth factor β (TGFβ) has no effect on myoblast proliferation, but is an inhibitor of muscle differentiation. Insulin-like growth factors (IGFs) have been shown to stimulate both myoblast proliferation and differentiation in rodents. Platelet derived growth factor (PDGF) is also mitogenic for myoblasts and is a potent inhibitor of muscle cell

differentiation see: Florini and Magri, 1989:256:C701-C711).

In vertebrate species both muscle tissue and neurons are potential sources of factors which stimulate myoblast proliferation and differentiation. In diseases affecting the neuromuscular system which are neural in origin (i.e., neurogenic), the muscle tissue innervated by the affected nerve becomes paralyzed and wastes progressively. During peripheral nerve regeneration and recovery from neurologic and myopathic disease, neurons may provide a source of growth factors which elicit the muscle regeneration described above and provide a mechanism for muscle recovery from wasting and atrophy.

A recently described family of growth factors, the neuregulins, are synthesized by motor neurons (Marchioni et al. *Nature* 362:313, 1993) and inflammatory cells (Tarakhovsky et al., *Oncogene* 6:2187-2196 (1991)). The neuregulins and related p185^{erbB2} binding factors have been purified, cloned and expressed (Benveniste et al., *PNAS* 82:3930-3934, 1985; Kimura et al., *Nature* 348:257-260, 1990; Davis and Stroobant, *J. Cell. Biol.* 110:1353-1360, 1990; Wen et al., *Cell* 69:559, 1992; Yarden and Ullrich, *Ann. Rev. Biochem.* 57:443, 1988; Holmes et al., *Science* 256:1205, 1992; Dobashi et al., *Proc. Natl. Acad. Sci.* 88:8582, 1991; Lupu et al., *Proc. Natl. Acad. Sci.* 89:2287, 1992). Recombinant neuregulins have been shown to be mitogenic for peripheral glia (Marchionni et al., Nature 362:313, 1993) and have been shown to influence the formation of the neuromuscular junction (Falls et al., Cell 72:801, 1993). Thus the regenerating neuron and the inflammatory cells associated with the recovery from neurogenic disease and nerve injury provide a source of factors which coordinate the remyelination of motor neurons and their ability to form the appropriate connection with their target. After

muscle has been reinnervat d th m t r neuron may provid factors to muscle, stimulating muscle growth and survival.

Currently, there is no useful therapy for the promotion of muscle differentiation and survival. Such a therapy would be useful for treatment of a variety of neural and muscular diseases and disorders.

Summary of the Invention

We have discovered that increased mitogenesis differentiation and survival of muscle cells may be achieved using proteins heretofore described as glial growth factors, acetylcholine receptor inducing activity (ARIA), heregulins, neu differentiation factor, and, more generally, neuregulins. We have discovered that these compounds are capable of inducing both the proliferation of muscle cells and the differentiation and survival of myotubes. These phenomena may occur in cardiac and smooth muscle tissues in addition to skeletal muscle tissues. Thus, the above compounds, regulatory compounds which induce synthesis of these compounds, and small molecules which mimic these compounds by binding to the receptors on muscle or by stimulating through other means the second messenger systems activated by the ligand-receptor complex are all extremely useful as prophylactic and affirmative therapies for muscle diseases.

A novel aspect of the invention involves the use of the above named proteins as growth factors to induce the mitogenesis, survival, growth and differentiation of muscle cells. Treating of the muscle cells to achieve these effects may be achieved by contacting muscle cells with a polypeptide described herein. The treatments may be provided to slow or halt net muscle loss or to

increase the amount or quality f muscle pr v nt in the vertebrate.

These factors may be used to produce muscle cell mitogenesis, differentiation, and survival in a vertebrate (preferably a mammal, more preferably a human) by administering to the vertebrate an effective amount of a polypeptide or a related compound. Neuregulin effects on muscle may occur, for example, by causing an increase in muscle performance by inducing the synthesis of particular isoforms of the contractile apparatus such as the myosin heavy chain slow and fast isoforms; by promoting muscle fiber survival via the induction of synthesis of protective molecules such as, but not limited to, dystrophin; and/or by increasing muscle innervation by, for example, increasing acetylcholine receptor molecules at the neuromuscular junction.

The term muscle cell as used herein refers to any cell which contributes to muscle tissue. Myoblasts, satellite cells, myotubes, and myofibril tissues are all included in the term "muscle cells" and may all be treated using the methods of the invention. Muscle cell effects may be induced within skeletal, cardiac and smooth muscles.

Mitogenesis may be induced in muscle cells, including myoblasts or satellite cells, of skeletal muscle, smooth muscle or cardiac muscle. Mitogenesis as used herein refers to any cell division which results in the production of new muscle cells in the patient. More specifically, mitogenesis in vitro is defined as an increase in mitotic index relative to untreated cells of 50%, more preferably 100%, and most preferably 300%, when the cells are exposed to labelling agent for a time equivalent to two doubling times. The mitotic index is the fraction of cells in the culture which have labelled

nuclei when grown in the presence of a tracer which only incorporates during S phase (i.e., BrdU) and the doubling time is defined as the average time required for the number of cells in the culture to increase by a factor of two).

An effect on mitogenesis in vivo is defined as an increase in satellite cell activation as measured by the appearance of labelled satellite cells in the muscle tissue of a mammal exposed to a tracer which only incorporates during S phase (i.e., BrdU). In useful therapeutic is defined in vivo as a compound which increases satellite cell activation relative to a control mammal by at least 10%, more preferably by at least 50%, and most preferably by more than 200% when the mammal is exposed to labelling agent for a period of greater than 15 minutes and tissues are assayed between 10 hours and 24 hours after administration of the mitogen at the therapeutic dose. Alternatively, satellite cell activation in vivo may be detected by monitoring the appearance of the intermediate filament vimentin by immunological or RNA analysis methods. When vimentin is assayed, the useful mitogen is defined as one which causes expression of detectable levels of vimentin in the muscle tissue when the therapeutically useful dosage is provided.

Myogenesis as used herein refers to any fusion of myoblasts to yield myotubes. Most preferably, an effect on myogenesis is defined as an increase in the fusion of myoblasts and the enablement of the muscle differentiation program. The useful myogenic therapeutic is defined as a compound which confers any increase in the fusion index in vitro. More preferably, the compound confers at least a 2.0-fold increase and, most preferably, the compound confers a 3-fold or greater

increase in the fusion index relative to the control. The fusion index is defined as the fraction of nuclei present in multinucleated cells in the culture relative to the total number of nuclei present in the culture. The percentages provided above are for cells assayed after 6 days of exposure to the myogenic compound and are relative to an untreated control. Myogenesis may also be determined by assaying the number of nuclei per area in myotubes or by measurement of the levels of muscle specific protein by Western analysis. Preferably, the compound confers at least a 2.0-fold increase in the density of myotubes using the assay provided, for example, herein, and, most preferably, the compound confers a 3-fold or greater increase.

The growth of muscle may occur by the increase in the fiber size and/or by increasing the number of fibers. The growth of muscle as used herein may be measured by A) an increase in wet weight, B) an increase in protein content, C) an increase in the number of muscle fibers, or D) an increase in muscle fiber diameter. An increase in growth of a muscle fiber can be defined as an increase in the diameter where the diameter is defined as the minor axis of ellipsis of the cross section. The useful therapeutic is one which increases the wet weight, protein content and/or diameter by 10% or more, more preferably by more than 50% and most preferably by more than 100% in an animal whose muscles have been previously degenerated by at least 10% and relative to a similarly treated control animal (i.e., an animal with degenerated muscle tissue which is not treated with the muscle growth compound). A compound which increases growth by increasing the number of muscle fibers is useful as a therapeutic when it increases the number of fibers in the diseased tissue by at least 1%, more preferably at least

20%, and most pr ferably, by at least 50%. These percentages are determined relative to the basal level in a comparable untreated undiseased mammal or in the contralateral undiseased muscle when the compound is administered and acts locally.

The survival of muscle fibers as used herein refers to the prevention of loss of muscle fibers as evidenced by necrosis or apoptosis or the prevention of other mechanisms of muscle fiber loss. Survival as used herein indicates an decrease in the rate of cell death of at least 10%, more preferably by at least 50%, and most preferably by at least 300% relative to an untreated control. The rate of survival may be measured by counting cells stainable with a dye specific for dead cells (such as propidium iodide) in culture when the cells are 8 days post-differentiation (i.e., 8 days after the media is changed from 20% to 0.5% serum).

Muscle regeneration as used herein refers to the process by which new muscle fibers form from muscle progenitor cells. The useful therapeutic for regeneration confers an increase in the number of new fibers by at least 1%, more preferably by at least 20%, and most preferably by at least 50%, as defined above.

The differentiation of muscle cells as used herein refers to the induction of a muscle developmental program which specifies the components of the muscle fiber such as the contractile apparatus (the myofibril). The therapeutic useful for differentiation increases the quantity of any component of the muscle fiber in the diseased tissue by at least 10% or more, more preferably by 50% or more, and most preferably by more than 100% relative to the equivalent tissue in a similarly treated control animal.

Atrophy of muscle as used herein refers to a significant loss in muscle fiber girth. By significant atrophy is meant a reduction of muscle fiber diameter in diseased, injured or unused muscle tissue of at least 10% relative to undiseased, uninjured, or normally utilized tissue.

Methods for treatment of diseases or disorders using the polypeptides or other compounds described herein are also part of the invention. Examples of muscular disorders which may be treated include skeletal muscle diseases and disorders such as myopathies, dystrophies, myoneural conductive diseases, traumatic muscle injury, and nerve injury. Cardiac muscle pathologies such as cardiomyopathies, ischemic damage, congenital disease, and traumatic injury may also be treated using the methods of the invention, as may smooth muscle diseases and disorders such as arterial sclerosis, vascular lesions, and congenital vascular diseases. For example, Duchennes muscular dystrophy, Beckkers' dystrophy, and Myasthenia gravis are but three of the diseases which may be treated using the methods of the invention.

The invention also includes methods for the prophylaxis or treatment of a tumor of muscle cell origin such as rhabdomyosarcoma. These methods include administration of an effective amount of a substance which inhibits the binding of one or more of the polypeptides described herein and inhibiting the proliferation of the cells which contribute to the tumor.

The methods of the invention may also be used to treat a patient suffering from a disease caused by a lack of a neurotrophic factor. By lacking a neurotrophic factor is meant a decreased amount of neurotrophic factor relative to an unaffected individual sufficient to cause

d tectable decrease in neuromuscular connections and/or muscular strength. The neurotrophic factor may be present at levels 10% below those observed in unaffected individuals. More preferably, the factor is present at levels 20% lower than are observed in unaffected individuals, and most preferably the levels are lowered by 80% relative to unaffected individuals under similar circumstances.

The methods of the invention make use of the fact that the neuregulin proteins are encoded by the same gene. A variety of messenger RNA splicing variants (and their resultant proteins) are derived from this gene and many of these products show binding to $P185^{ergB2}$ and activation of the same. Products of this gene have been used to show muscle cell mitogenic activity (see Examples 1 and 2, below), differentiation (Examples 3 and 6), and survival (Examples 4 and 5). This invention provides a use for all of the known products of the neuregulin gene (described herein and in the references listed above) which have the stated activities as muscle cell mitogens, differentiation factors, and survival factors. Most preferably, recombinant human GGF2 (rhGGF2)is used in these methods.

The invention also relates to the use of other, not yet naturally isolated, splicing variants of the neuregulin gene. Fig. 29 shows the known patterns of splicing. These patterns are derived from polymerase chain reaction experiments (on reverse transcribed RNA), analysis of cDNA clones (as presented within), and analysis of published sequences encoding neuregulins (Peles et al., Cell 69:205 (1992) and Wen et al., Cell 69:559 (1992)). These patterns, as well as additional patterns disclosed herein, represent probable splicing variants which exist. The splicing variants are fully

described in Goodearl et al., USSN 08/036,555, filed March 24, 1993, incorporated herein by reference.

More specifically, cell division, survival, differentiation and growth of muscle cells may be achieved by contacting muscle cells with a polypeptide defined by the formula

WBAZCX

wherein WBAZCX is composed of the polypeptide segments shown in Fig. 30 (SEQ ID Nos. 132, 134, 135, 137-139, 156); wherein W comprises the polypeptide segment F, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprises the polypeptide segment C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL, or C/D C/D' D' HKL and/or by contacting muscle cells with a polypeptide defined by the formula

YBAZCX

wherein YBAZCX is composed of the polypeptide segments shown in Fig. 30 (SEQ ID Nos. 133-135, 156, 159); wherein Y comprises the polypeptide segment E, or is absent; wherein Z comprises the polypeptide segment G or is absent; and wherein X comprises the polypeptide segment C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL, or C/D C/D' D' HKL.

Generally, the N-terminus of the above-described polypeptides begins with either the F or E polypeptide

segments. When the F p lyp ptide is present it may be cleaved upon maturation of the protein to yield the mature polypeptide. When the E sequence is present the first 50 amino acids which represent the N-terminal signal sequence may be absent from the polypeptides.

Furthermore, the invention includes a method of treating muscle cells by the application to the muscle cell of a

-30 kD polypeptide factor isolated from the MDA-MB 231 human breast cell line; or

-35 kD polypeptide factor isolated from the rat I-EJ transformed fibroblast cell line to the glial cell or

-75 kD polypeptide factor isolated from the SKBR-3 human breast cell line; or

-44 kD polypeptide factor isolated from the rat I-EJ transformed fibroblast cell line; or

-25 kD polypeptide factor isolated from activated mouse peritoneal macrophages; or

-45 kD polypeptide factor isolated from the MDA - MB 231 human breast cell; or

-7 to 14 kD polypeptide factor isolated from the ATL-2 human T-cell line to the glial cell; or

-25 kD polypeptide factor isolated from the bovine kidney cells; or

-42 kD ARIA polypeptide factor isolated from brain; -46-47 kD polypeptide factor which stimulates 0-2A glial progenitor cells; or

-43-45 kD polypeptide factor, GGFIII,175 U.S. patent application Serial No. 07/931,041, filed August 17, 1992, incorporated herein by reference.

The invention further includes methods for the use of the EGFL1, EGFL2, EGFL3, EGFL4, EGFL5, and EGFL6 polypeptides, Fig. 37 to 42 and SEQ ID Nos. 150 to 155,

r spectively, f r th treatment of muscl cells in vivo and in vitro.

Also included in the invention is the administration of the GGF2 polypeptide whose sequence is shown in Fig. 44 for the treatment of muscle cells.

An additional important aspect of the invention are methods for treating muscle cells using:

(a) a basic polypeptide factor also known to have glial cell mitogenic activity, in the presence of fetal calf plasma, a molecular weight of from about 30 kD to about 36 kD, and including within its amino acid sequence any one or more of the following peptide sequences:

F K G D A H T E (SEQ ID NO: 1)
A S L A D E Y E Y M X K (SEQ ID NO: 2)
T E T S S S G L X L K (SEQ ID NO: 3)
A S L A D E Y E Y M R K (SEQ ID NO: 7)
A G Y F A E X A R (SEQ ID NO: 11)
T T E M A S E Q G A (SEQ ID NO:13)
A K E A L A A L K (SEQ ID NO: 14)
F V L Q A K K (SEQ ID NO: 15)
E T Q P D P G Q I L K K V P M V I G A Y T (SEQ ID NO: 165)
E Y K C L K F K W F K K A T V M (SEQ ID NO: 17)
E X K F Y V P (SEQ ID NO: 19)
K L E F L X A K (SEQ ID NO: 32); and

(b) a basic polypeptide factor for use in treating muscle cells which is also known to stimulate glial cell mitogenesis in the presence of fetal calf plasma, has a molecular weight of from about 55 kD to about 63 kD, and including within its amino acid sequence any one or more of the following peptide sequences:

V H Q V W A A K	(SEQ ID NO: 33)
Y I P P M E P E A X S S G	(SEQ ID NO: 34)
L G A W G P P A F P V X Y	(SEQ ID NO: 35)
W F V V I E G K	(SEQ ID NO: 36)
A S P V S V G S V Q E L Q R	(SEQ ID NO: 37)
V C L L T V A A L P P T	(SEQ ID NO: 38)
K V H Q V W A A K	(SEQ ID NO: 48)
K A S L A D S G E Y M X K	(SEQ ID NO: 49)
D L L L X V	(SEQ ID NO: 39)

Methods for the use of the peptide sequences set out above, derived from the smaller molecular weight polypeptide factor, and from the larger molecular weight polypeptide factor, are also aspects of this invention. Monoclonal antibodies to the above peptides are themselves useful investigative tools and therapeutics.

Thus, the invention further embraces methods of using a polypeptide factor having activities useful for treating muscle cells and including an amino acid sequence encoded by:

(a) a DNA sequence shown in any one of Figs. 27A, 27B or 27C, SEQ ID Nos. 129-131, respectively;

(b) a DNA sequence shown in Fig. 21, SEQ ID No. 85;

(c) the DNA sequence represented by nucleotides 281-557 of the sequence shown in Fig. 27A, SEQ ID No. 129; or

(d) a DNA sequence hybridizable to any one of the DNA sequences according to (a), (b) or (c).

Following factors as muscle cell mitogens:

(a) a basic polypeptide factor which has, if obtained from bovine pituitary material, an observed molecular weight, whether in reducing conditions or not, of from about 30kD to about 36kD on SDS-polyacrylamide

gel electrophoresis which factor has muscle cell mitogenic activity including stimulating the division of myoblasts, and when isolated using reversed-phase HPLC retains at least 50% of said activity after 10 weeks incubation in 0.1% trifluoroacetic acid at 4°C; and

(b) a basic polypeptide factor which has, if obtained from bovine pituitary material, an observed molecular weight, under non-reducing conditions, of from about 55 kD to about 63 Kd on SDS-polyacrylamide gel electrophoresis which factor the human equivalent of which is encoded by DNA clone GGF2HBS5 and which factor has muscle cell mitogenic activity and when isolated using reversed-phase HPLC retains at least 50% of the activity after 4 days incubation in 0.1% trifluoroacetic acid at 4°C.

Thus other important aspects of the invention are the use of:

(a) A series of human and bovine polypeptide factors having cell mitogenic activity including stimulating the division of muscle cells. These peptide sequences are shown in Figs. 30, 31, 32 and 33, SEQ ID Nos. 132-133, respectively.

(b) A series of polypeptide factors having cell mitogenic activity including stimulating the division of muscle cells and purified and characterized according to the procedures outlined by Lupu et al. Science 249: 1552 (1990); Lupu et al. Proc. Natl. Acad. Sci USA 89: 2287 (1992); Holmes et al. Science 256: 1205 (1992); Peles et al. 69: 205 (1992); Yarden and Peles Biochemistry 30: 3543 (1991); Dobashi et al. Proc. Natl. Acad. Sci. 88: 8582 (1991); Davis et al. Biochem. Biophys. Res. Commun. 179: 1536 (1991); Beaumont et al., patent application PCT/US91/03443 (1990); Bottenstein, U.S. Patent No.

5,276,145, issu d 1/4/94; and Green et al. patent application PCT/US91/02331 (1990).

(c) A polypeptide factor (GGFBPP5) having glial cell mitogenic activity including stimulating the division of muscle cells. The amino acid sequence is shown in Fig. 31, SEQ ID No. 144.

Methods for stimulating mitogenesis of a myoblast by contacting the myoblast cell with a polypeptide defined above as a muscle cell mitogen *in vivo* or *in vitro* are included as features of the invention.

Muscle cell treatments may also be achieved by administering DNA encoding the polypeptide compounds described above in an expressible genetic construction. DNA encoding the polypeptide may be administered to the patient using techniques known in the art for delivering DNA to the cells. For example, retroviral vectors, electroporation or liposomes may be used to deliver DNA.

The invention includes the use of the above named family of proteins as extracted from natural sources (tissues or cell lines) or as prepared by recombinant means.

Other compounds in particular, peptides, which bind specifically to the $p185^{erbB2}$ receptor can also be used according to the invention as muscle cell mitogens. A candidate compound can be routinely screened for $p185^{erbB2}$ binding, and, if it binds, can then be screened for glial cell mitogenic activity using the methods described herein.

The invention includes use of any modifications or equivalents of the above polypeptide factors which do not exhibit a significantly reduced activity. For example, modifications in which amino acid content or sequence is altered without substantially adversely affecting activity are included. The statements of effect and use

contained herein are therefore to be construed accordingly, with such uses and effects employing modified or equivalent factors being part of the invention.

The human peptide sequences described above and presented in Figs. 30, 31, 32 and 33, SEQ ID Nos. 132-146, respectively, represent a series of splicing variants which can be isolated as full length complementary DNAs (cDNAS) from natural sources (cDNA libraries prepared from the appropriate tissues) or can be assembled as DNA constructs with individual exons (e.g., derived as separate exons) by someone skilled in the art.

The invention also includes a method of making a medicament for treating muscle cells, i.e., for inducing muscular mitogenesis, myogenesis, differentiation, or survival, by administering an effective amount of a polypeptide as defined above. Such a medicament is made by administering the polypeptide with a pharmaceutically effective carrier.

Another aspect of the invention is the use of a pharmaceutical or veterinary formulation comprising any factor as defined above formulated for pharmaceutical or veterinary use, respectively, optionally together with an acceptable diluent, carrier or excipient and/or in unit dosage form. In using the factors of the invention, conventional pharmaceutical or veterinary practice may be employed to provide suitable formulations or compositions.

Thus, the formulations to be used as a part of the invention can be applied to parenteral administration, for example, intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal,

intraperit neal, topical, intranasal, a r sol, scarification, and also oral, buccal, rectal or vaginal administration.

The formulations of this invention may also be administered by the transplantation into the patient of host cells expressing the DNA encoding polypeptides which are effective for the methods of the invention or by the use of surgical implants which release the formulations of the invention.

Parenteral formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are to be found in, for example, "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain as excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes, biocompatible, biodegradable lactide polymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present factors. Other potentially useful parenteral delivery systems for the factors include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration,

methoxysalicylate for rectal administration, or citric acid for vaginal administration.

The present factors can be used as the sole active agents, or can be used in combination with other active ingredients, e.g., other growth factors which could facilitate neuronal survival in neurological diseases, or peptidase or protease inhibitors.

The concentration of the present factors in the formulations of the invention will vary depending upon a number of issues, including the dosage to be administered, and the route of administration.

In general terms, the factors of this invention may be provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for parenteral administration. General dose ranges are from about 1 mg/kg to about 1 g/kg of body weight per day; a preferred dose range is from about 0.01 mg/kg to 100 mg/kg of body weight per day. The preferred dosage to be administered is likely to depend upon the type and extent of progression of the pathophysiological condition being addressed, the overall health of the patient, the make up of the formulation, and the route of administration.

The polypeptide factors utilized in the methods of the invention can also be used as immunogens for making antibodies, such as monoclonal antibodies, following standard techniques. These antibodies can, in turn, be used for therapeutic or diagnostic purposes. Thus, conditions perhaps associated with muscle diseases resulting from abnormal levels of the factor may be tracked by using such antibodies. *In vitro* techniques can be used, employing assays on isolated samples using standard methods. Imaging methods in which the antibodies are, for example, tagged with radioactive isotopes which can be imaged outside the body using

t chniques f r th art of tumor imaging may also be employed.

A further general aspect of the invention is the use of a factor of the invention in the manufacture of a medicament, preferably for the treatment of a muscular disease or disorder. The "GGF2" designation is used for all clones which were previously isolated with peptide sequence data derived from GGF-II protein (i.e., GGF2HBS5, GGF2BPP3) and, when present alone (i.e., GGF2 or rhGGF2), to indicate recombinant human protein encoded by plasmids isolated with peptide sequence data derived from the GGF-II protein (i.e., as produced in insect cells from the plasmid HBS5). Recombinant human GGF from the GGFHBS5 clone is called GGF2, rhGGF2 and GGF2HBS5 polypeptide.

Treating as used herein means any administration of the compounds described herein for the purpose of increasing muscle cell mitogenesis, survival, and/or differentiation, and/or decreasing muscle atrophy and degeneration. Most preferably, the treating is for the purpose of reducing or diminishing the symptoms or progression of a disease or disorder of the muscle cells. Treating as used herein also means the administration of the compounds for increasing or altering the muscle cells in healthy individuals. The treating may be brought about by the contacing of the muscle cells which are sensitive or responsive to the compounds described herein with an effective amount of the compound, as described above. Inhibitors of the compounds described herein may also be used to halt or slow diseases of muscle cell proliferation.

Brief Description of the Drawings

The drawings will first be described.

Drawings

Fig. 1 is a graph showing the results of rhGGF2 in a myoblast mitogenesis assay.

Fig. 2 is a graph showing the effect of rhGGF2 on the number of nuclei in myotubes.

Fig. 3 is a graph of a survival assay showing the effect of rhGGF2 on survival of differentiated myotubes.

Fig. 4 is a graph of survival assays showing the effect of rhGGF2 on differentiated myotubes relative to human platelet derived growth factor, human fibroblast growth factor, human epidermal growth factor, human leucocyte inhibitory factor, and human insulin-like growth factors I and II.

Fig. 5 is a graph showing the increased survival on Duchenne muscular dystrophy cells in the presence of rhGGF2. Fig. 6 is a graph of increasing human growth hormone (hGH) expression in C2 cells from an hGH reporter gene under control of the AchR delta subunit transcriptional control elements. This increase is tied to the addition of GGF2 to the media.

Fig. 7 is a graph of increasing hGH reporter synthesis and bungarotoxin (BTX) binding to AchRs following the addition of increasing amounts of GGF2 to C2 cells.

Figs. 8, 9, 10 and 11 are the peptide sequences derived from GGF-I and GGF-II, SEQ ID Nos. 1-20, 22-29, 32-50 and 165, (see Examples 11-13 hereinafter).

Fig. 9, Panel A, is the sequences of GGF-I peptides used to design degenerate oligonucleotide probes and degenerate PCR primers are listed (SEQ ID Nos. 1, 17 and 22-29). Some of the sequences in Panel A were also used to design synthetic peptides. Panel B is a listing

of the sequences of novel peptid s that were too short (less than 6 amino acids) for the design of degenerate probes or degenerate PCR primers (SEQ ID Nos. 17 and 32);

Fig. 11, Panel A, is a listing of the sequences of GGF-II peptides used to design degenerate oligonucleotide probes and degenerate PCR primers (SEQ ID Nos. 42-49). Some of the sequences in Panel A were used to design synthetic peptides. Panel B is a listing of the novel peptide that was too short (less than 6 amino acids) for the design of degenerate probes or degenerate PCR primers (SEQ ID No. 50);

Figs. 12, 13A, 13B, 14, 15, 16, 17, 18, and 19 relate to Example 8, below, and depict the mitogenic activity of factors of the invention;

Figs. 20, 21, 22, 23, 24, 25, 26, and 27 relate to Example 10, below and are briefly described below:

Fig. 20 is a listing of the degenerate oligonucleotide probes (SEQ ID Nos. 51-84) designed from the novel peptide sequences in Figure 7, Panel A and Figure 9, Panel A;

Fig. 21 (SEQ ID No. 85) depicts a stretch of the putative bovine GGF-II gene sequence from the recombinant bovine genomic phage GGF2BG1, containing the binding site of degenerate oligonucleotide probes 609 and 650 (see Figure 18, SEQ ID NOs. 66 and 69, respectively). The figure is the coding strand of the DNA sequence and the deduced amino acid sequence in the third reading frame. The sequence of peptide 12 from factor 2 (bold) is part of a 66 amino acid open reading frame (nucleotides 75272);

Fig. 22 is the degenerate PCR primers (Panel A, SEQ IS Nos. 86-104) and unique PCR primers (Panel B, SEQ ID Nos. 105-115) used in experiments to isolate segments of the bovine GGF-II coding sequences present in RNA from posterior pituitary;

Fig. 23 depicts of the nine distinct contiguous bovine GGF-II cDNA structures and sequences that were obtained in PCR amplification experiments. The top line of the Figure is a schematic of the coding sequences which contribute to the cDNA structures that were characterized;

Fig. 24 is a physical map of bovine recombinant phage of GGF2BG1. The bovine fragment is roughly 20 kb in length and contains two exons (bold) of the bovine GGF-II gene. Restriction sites for the enzymes Xbal, SpeI, Ndel, EcoRI, Kpnl, and SstI have been placed on this physical map. Shaded portions correspond to fragments which were subcloned for sequencing;

Fig. 25 is a schematic of the structure of three alternative gene products of the putative bovine GGF-II gene. Exons are listed A through E in the order of their discovery. The alternative splicing patterns 1, 2 and 3 generate three overlapping deduced protein structures (GGF2BPP1, 2, and 3), which are displayed in the various Figs. 27A, 27B, 27C (described below);

Fig. 26 (SEQ ID Nos. 116-128) is a comparison of the GGF-I and GGF-II sequences identified in the deduced protein sequences shown in Figs. 27A, 27B, 27C (described below) with the novel peptide sequences listed in Figs. 9 and 11. The Figure shows that six of the nine novel GGF-II peptide sequences are accounted for in these deduced protein sequences. Two peptide sequences similar to GGF-I sequences are also found;

Fig. 27 (SEQ ID No. 129) is a listing of the coding strand DNA sequence and deduced amino acid sequence of the cDNA obtained from splicing pattern number 1 in Figure 25. This partial cDNA of the putative bovine GGF-II gene encodes a protein of 206 amino acids in length. Peptides in bold were those identified from

th lists presented in Figs. 9 and 11. P tential glycosylation sites are underlined (along with polyadenylation signal AATAAA);

Fig. 27 (SEQ ID No. 130) is a listing of the coding strand DNA sequence and deduced amino acid sequence of the cDNA obtained from splicing pattern number 2 in Fig. 25. This partial cDNA of the putative bovine GGF-II gene encodes a protein of 281 amino acids in length. Peptides in bold are those identified from the lists presented in Figs. 7 and 9. Potential glycosylation sites are underlined (along with polyadenylation signal AATAAA);

Fig. 27 (SEQ ID No. 131) is a listing of the coding strand DNA sequence and deduced amino acid sequence of the cDNA obtained from splicing pattern number 3 in Fig. 25. This partial cDNA of the putative bovine GGF-II gene encodes a protein of 257 amino acids in length. Peptides in bold are those identified from the lists in Figs. 9 and 11. Potential glycosylation sites are underlined (along with polyadenylation signal AATAAA).

Fig. 28, which relates to Example 16 hereinafter, is an autoradiogram of a cross hybridization analysis of putative bovine GGF-II gene sequences to a variety of mammalian DNAs on a southern blot. The filter contains lanes of EcoRI-digested DNA (5 μg per lane) from the species listed in the Figure. The probe detects a single strong band in each DNA sample, including a four kilobase fragment in the bovine DNA as anticipated by the physical map in Fig. 24. Bands of relatively minor intensity are observed as well, which could represent related DNA sequences. The strong hybridizing band from each of the other mammalian DNA samples presumably represents the GGF-II homologue of those species.

Fig. 29 is a diagram of representative splicing variants. The coding segments are represented by F, E, B, A, G, C, C/D, C/D', D, D', H, K and L. The location of the peptide sequences derived from purified protein are indicated by "o".

Fig. 30 (SEQ ID Nos. 136-143, 156, 157, 169-178) is a listing of the DNA sequences and predicted peptide sequences of the coding segments of GGF. Line 1 is a listing of the predicted amino acid sequences of bovine GGF, line 2 is a listing of the nucleotide sequences of bovine GGF, line 3 is a listing of the nucleotide sequences of human GGF (heregulin) (nucleotide base matches are indicated with a vertical line) and line 4 is a listing of the predicted amino acid sequences of human GGF/heregulin where it differs from the predicted bovine sequence. Coding segments E, A' and K represent only the bovine sequences. Coding segment D' represents only the human (heregulin) sequence.

Fig. 31 (SEQ ID No. 144) is the predicted GGF2 amino acid sequence and nucleotide sequence of BPP5. The upper line is the nucleotide sequence and the lower line is the predicted amino acid sequence.

Fig. 32 (SEQ ID No. 145) is the predicted amino acid sequence and nucleotide sequence of GGF2BPP2. The upper line is the nucleotide sequence and the lower line is the predicted amino acid sequence.

Fig. 33 (SEQ ID No. 146) is the predicted amino acid sequence and nucleotide sequence of GGF2BPP4. The upper line is the nucleotide sequence and the lower line is the predicted amino acid sequence.

Fig. 34 (SEQ ID Nos. 147-149) depicts the alignment of two GGF peptide sequences (GGF2BPP4 and GGF2BPP5) with the human EGF (hEGF). Asterisks indicate positions of conserved cysteines.

Fig. 35 depicts the 1 v 1 of GGF activity (Schwann cell mitogenic assay) and tyrosine phosphorylation of a ca. 200kD protein (intensity of a 200 kD band on an autoradiogram of a Western blot developed with an antiphosphotyrosine polyclonal antibody) in response to increasing amounts of GGF.

Fig. 36 is a list of splicing variants derived from the sequences shown in Fig. 30.

Fig. 37 is the predicted amino acid sequence, bottom, and nucleic sequence, top, of EGFL1 (SEQ ID No. 150).

Fig. 38 is the predicted amino acid sequence, bottom, and nucleic sequence, top, of EGFL2 (SEQ ID No. 151).

Fig. 39 is the predicted amino acid sequence, bottom, and nucleic sequence, top, of EGFL3 (SEQ ID No. 152).

Fig. 40 is the predicted amino acid sequence, bottom, and nucleic sequence, top, of EGFL4 (SEQ ID No. 153).

Fig. 41 is the predicted amino acid sequence, bottom, and nucleic sequence, top, of EGFL5 (SEQ ID No. 154).

Fig. 42 is the predicted amino acid sequence, bottom, and nucleic sequence, top, of EGFL6 (SEQ ID No. 159).

Fig. 43 is a scale coding segment map of the clone. T3 refers to the bacteriophage promoter used to produce mRNA from the clone. R = flanking EcoRI restriction enzyme sites. 5' UT refers to the 5' untranslated region. E, B, A, C, C/D', and D refer to the coding segments. O = the translation start site. Λ = the 5' limit of the region homologous to the bovine E

s gment (see Example 17) and 3′ UT refers t th 3′ untranslated region.

Fig. 44 is the predicted amino acid sequence (middle) and nucleic sequence (top) of GGF2HBS5 (SEQ ID No. 21). The bottom (intermittent) sequence represents peptide sequences derived from GGF-II preparations (see Figs. 8, 9).

Fig. 45 (A) is a graph showing the purification of rGGF on cation exchange column by fraction; Fig. 45 (B) is a photograph of a Western blot using fractions as depicted in (A) and a GGFII specific antibody.

Fig. 46 is the sequence of the GGFHBS5, GGFHFB1 and GGFBPP5 polypeptides (SEQ ID NOS: 166, 167, and 168).

Fig. 47 is a map of the plasmid pcDHRFpolyA.

Detailed Description

The invention pertains to the use of isolated and purified neuregulin factors and DNA sequences encoding these factors, regulatory compounds which increase the extramuscular concentrations of these factors, and compounds which are mimetics of these factors for the induction of muscle cell mitogenesis, differentiation, and survival of the muscle cells *in vivo* and *in vitro*.

It is evident that the gene encoding GGF/p185^{erbB2} binding neuregulin proteins produces a number of variably-sized, differentially-spliced RNA transcripts that give rise to a series of proteins. These proteins are of different lengths and contain some common peptide sequences and some unique peptide sequences. The conclusion that these factors are encoded by a single gene is supported by the differentially-spliced RNA sequences which are recoverable from bovine posterior pituitary and human breast cancer cells (MDA-MB-231)). Further support for this conclusion derives from the size

rang of proteins which act as both mitogens for muscle tissue (as disclosed herein) and as ligands for the $p185^{erbB2}$ receptor (see below).

Further evidence to support the fact that the genes encoding GGF/$p185^{erbB2}$ binding proteins are homologous comes from nucleotide sequence comparison. Holmes et al., (Science 256:1205-1210, 1992) demonstrate the purification of a 45-kilodalton human protein (Heregulin-α) which specifically interacts with the receptor protein $p185^{erbB2}$. Peles et al. (Cell 69:205 (1992)) and Wen et al. (Cell 69:559 (1992)) describe a complementary DNA isolated from rat cells encoding a protein called "neu differentiation factor" (NDF). The translation product of the NDF cDNA has $p185^{erbB2}$ binding activity. Several other groups have reported the purification of proteins of various molecular weights with $p185^{erbB2}$ binding activity. These groups include Lupu et al. ((1992) Proc. Natl. Acad. Sci. USA 89:2287); Yarden and Peles ((1991) Biochemistry 30:3543); Lupu et al. ((1990) Science 249:1552)); Dobashi et al. ((1991) Biochem. Biophys. Res. Comm. 179:1536); and Huang et al. ((1992) J. Biol. Chem. 257:11508-11512).

We have found that $p185^{erbB2}$ receptor binding proteins stimulate muscle cell mitogenesis and hence, stimulates myotube formation (myogenesis). This stimulation results in increased formation of myoblasts and increased formation of myotubes (myogenesis). The compounds described herein also stimulate increased muscle growth, differentiation, and survival of muscle cells. These ligands include, but are not limited to the GGF's, the neuregulins, the heregulins, NDF, and ARIA. As a result of this mitogenic activity, these proteins, DNA encoding these proteins, and related compounds may be administered to patients suffering from traumatic damage

r diseases f th muscle tissu . It is understood that all methods provided for the purpose of mitogenesis are useful for the purpose of myogenesis. Inhibitors of these ligands (such as antibodies or peptide fragments) may be administered for the treatment of muscle derived tumors.

These compounds may be obtained using the protocols described herein (Examples 9-17) and in Holmes et al., *Science* 256: 1205 (1992); Peles et al., *Cell* 69:205 (1992); Wen et al., *Cell* 69:559 (1992); Lupu et al., *Proc. Natl. Acad. Sci. USA* 89:2287 (1992); Yarden and Peles, *Biochemistry* 30:3543 (1991); Lupu et al., *Science* 249:1552 (1990); Dobashi et al., *Biochem. Biophys. Res. Comm.* 179:1536 (1991); Huang et al., *J. Biol. Chem.* 257:11508-11512 (1992); Marchionni et al., *Nature* 362:313, (1993); and in the GGF-III patent, all of which are incorporated herein by reference. The sequences are provided and the characteristics described for many of these compounds. For sequences see Figs. 8-11, 20-27C, 29-34, 36-44, and 46. For protein characteristics see Figs. 12-19, 28 35, 45A and 45B.

Compounds may be assayed for their usefulness in vitro using the methods provided in the examples below. In vivo testing may be performed as described in Example 1 and in Sklar et al., In Vitro Cellular and Developmental Biology 27A:433-434, 1991.

Other Embodiments

The invention includes methods for the use of any protein which is substantially homologous to the coding segments in Fig. 30 (SEQ ID Nos. 132-143, 156, 1576-147, 160, and 161) as well as other naturally occurring GGF polypeptides for the purpose of inducing muscle mitogenesis. Also included are the use of: allelic

variations; natural mutants; induc d mutants; pr teins encoded by DNA that hybridizes under high or low stringency conditions to a nucleic acid naturally occurring (for definitions of high and low stringency see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, 6.3.1 - 6.3.6, hereby incorporated by reference); and the use of polypeptides or proteins specifically bound by antisera to GGF polypeptides. The term also includes the use of chimeric polypeptides that include the GGF polypeptides comprising sequences from Fig. 28 for the induction of muscle mitogenesis.

As will be seen from Example 8, below, the present factors exhibit mitogenic activity on a range of cell types. The general statements of invention above in relation to formulations and/or medicaments and their manufacture should clearly be construed to include appropriate products and uses.

A series of experiments follow which provide additional basis for the claims described herein. The following examples relating to the present invention should not be construed as specifically limiting the invention, or such variations of the invention, now known or later developed.

The examples illustrate our discovery that recombinant human GGF2 (rhGGF2) confers several effects on primary human muscle culture. rhGGF2 has significant effects in three independent biological activity assays on muscle cultures. The polypeptide increased mitogenesis as measured by proliferation of subconfluent quiescent myoblasts, increased differentiation by confluent myoblasts in the presence of growth factor, and increased survival of differentiated myotubes as measured by loss of dye exclusion and increased acetylcholine receptor synthesis. These activities indicate efficacy

of GGF2 and other neuregulins in inducing muscle repair, regeneration, and prophylactic effects on muscle degeneration.

EXAMPLE 1

Mitogenic Activity of rhGGF on Myoblasts

Clone GGF2HBS5 was expressed in recombinant Baculovirus infected insect cells as described in Example 14, infra, and the resultant recombinant human GGF2 was added to myoblasts in culture (conditioned medium added at 40 μl/ml). Myoblasts (057A cells) were grown to preconfluence in a 24 well dish. Medium was removed and replaced with DMEM containing 0.5% fetal calf serum with or without GGF2 conditioned medium at a concentration of 40 μl/ml. Medium was changed after 2 days and cells were fixed and stained after 5 days. Total nuclei were counted as were the number of nuclei in myoblasts (Table 1).

TABLE 1

Treatment	Total Number of Nuclei/mm^2	Nuclei in Myotubes	Fusion Index
Control	395 ± 28.3	204 ± 9.19	0.515 ± 0.01
GGF 40μl/ml	636 ± 8.5	381 ± 82.7	0.591 ± 0.15

GGF treated myoblasts showed an increased number of total nuclei (636 nuclei) over untreated controls (395 nuclei) indicating mitogenic activity. rhGGF2 treated myotubes had a greater number of nuclei (381 nuclei) than untreated controls (204 nuclei). Thus, rhGGF2 enhances the total number of nuclei through proliferation and increased cell survival. rhGGF2 is also likely to enhance the formation of myotubes.

The mitog nic activity of rhGGF2 may be measured *in vivo* by giving a continuous supply of GGF2 and [^{3}H]thymidine to rat muscle via an osmotic mini pump. The muscle bulk is determined by wet weight after one and two weeks of treatment. DNA replication is measured by counting labeled nuclei in sections after coating for autoradiography (Sklar et al., In Vitro Cellular and Developmental Biology 27A:433-434, 1991) in sham and rhGGF2-treated muscle. Denervated muscle is also examined in this rat animal model via these methods and this method allows the assessment of the role of rhGGF2 in muscle atrophy and repair. Mean fiber diameter can also be used for assessing effects of FGF on prevention of atrophy.

EXAMPLE 2

Effect of rhGGF2 on Muscle Cell Mitogenesis

Quiescent primary clonal human myoblasts were prepared as previously described (Sklar, R., Hudson, A., Brown, R., *In vitro Cellular and Developmental Biology* 1991; 27A:433-434). The quiescent cells were treated with the indicated agents (rhGGF2 conditioned media, PDGF with and without methylprednisolone, and control media) in the presence of 10μM BrdU, 0.5% FCS in DMEM. After two days the cells were fixed in 4% paraformaldehyde in PBS for 30 minutes, and washed with 70% ethanol. The cells were then incubated with an anti-BrdU antibody, washed, and antibody binding was visualized with a peroxidase reaction. The number of staining nuclei were then quantified per area. The results show that GGF2 induces an increase in the number of labelled nuclei per area over controls (see Table 2).

TABLE 2

Mitogenic Effects of GGF on Human Myoblasts

Treatment	Labelled Nuclei/cm^2	T-Test p value
Control	120 ± 22.4	
Infected Control	103 ± 11.9	
GGF 5 µl/ml	223 ± 33.8	0.019
PDGF 20 ng/ml	418 ± 45.8	0.0005
IGFI 30 ng/ml	280 ± 109.6	0.068
Methylprednisolone 1.0 µM	142 ± 20.7	0.293

Platelet derived growth factor (PDGF) was used as a positive control. Methylprednisolone (a corticosteroid) was also used in addition to rhGGF2 and showed no significant increase in labelling of DNA.

rhGGF2 purified to homogeneity (>95% pure) is also mitogenic for human myoblasts (Fig. 1).

Recombinant human GGF2 also causes mitogenesis of primary human myoblasts (see Table 2 and Fig. 1). The mitogenesis assay is performed as described above. The mitotic index is then calculated by dividing the number of BrdU positive cells by the total number of cells.

EXAMPLE 3

Effect of rhGGF2 on Muscle Cell Differentiation

The effects of purified rhGGF2 (95% pure) on muscle culture differentiation were examined (Fig. 2). Confluent myoblast cultures were induced to differentiate by lowering the serum content of the culture medium from 20% to 0.5%. The test cultures were treated with the indicated concentration of rhGGF2 for six days, refreshing the culture medium every 2 days. The cultures

wer then fixed, stained, and the number of nucl i counted per millimeter. The data in Fig. 2 demonstrate a large increase in the number of nuclei in myotubes when rhGGF2 is present, relative to controls.

EXAMPLE 4

Effect of rhGGF2 on the Survival of Differentiated Myotubes

The survival of differentiated myotubes was significantly increased by rhGGF2 treatment. Muscle cultures were differentiated in the presence of rhGGF2 and at various times the number of dead myotubes were counted by propidium iodide staining. As can be seen in Fig. 3, the number of dead myotubes is lower in the rhGGF2 treated culture at 4, 5, 6, and 8 days of differentiation. The number of nuclei in myotubes was significantly increased by GGF2 treatment compared to untreated cultures after 8 days of differentiation. Specifically, the control showed 8.6 myonuclei/mm^2, while rhGGF2 treated cultures showed 57.2 myonuclei/mm^2 ($p=0.035$) when counted on the same plates after geimsa staining.

The survival assay was also performed with other growth factors which have known effects on muscle culture. The rhGGF2 effect was unique among the growth factors tested (Fig. 4). In this experiment cultures were treated in parallel with the rhGGF2 treated plates with the indicated concentrations of the various growth factors. Survival of myotubes was measured as above at 8 days of differentiation of 057A myoblast cells. Concentrations of factors were as follows: rhGGF2: 100ng/ml; human platelet derived growth factor: 20ng/ml; human basic fibroblast growth factor: 25ng/ml; human epidermal growth factor: 30ng/ml; human leucocyte

inhibitory factor: 10ng/ml; human insulin like growth factor I: 30ng/ml; human insulin like growth factor II: 25ng/ml.

The observed protection of differentiated myotubes from death indicates that rhGGF2 has promise as a therapy for intervention of muscle degeneration characterized by numerous muscle diseases. Thus, agents which increase the extramuscular concentration of neuregulins may have a prophylactic effect or slow the progress of muscle-wasting disorders and increase rates of muscle differentiation, repair, conditioning, and regeneration.

EXAMPLE 5

rhGGF2 Promotes Survival of Differentiated Myotubes with a Genetic Defect at the Duchenne Muscular Dystrophy Locus

The positive effects of rhGGF2 on myotube survival could reflect potential efficacy in degenerative disorders. These effects on myotube survival were tested on a clonally-derived primary Duchenne myoblast to determine if the response observed in normal muscle culture could also be demonstrated in cultures derived from diseased individuals. The data presented in Fig. 5 was obtained using the same muscle culture conditions (Example 4, above) used for normal individual. rhGGF2 significantly decreased the number of dead myotubes in the differentiated Duchenne muscle culture, compared to controls ($p=0.032$). Concentrations were as follows: GGF2: 100ng/ml; human platelet derived growth factor: 20ng/ml; human insulin like growth factor I: 30ng/ml.

This example demonstrates that rhGGF2 can also promote survival of differentiated Duchenne myotubes and provides strong evidence that rhGGF2 may slow or prevent the course of muscle degeneration and wasting in mammals.

EXAMPLE 6

rhGGF2 Effect on the Differentiation Program: Induction of MHC Slow and Dystrophin Proteins

The effects of purified rhGGF2 on muscle culture differentiation was also examined by Western analysis of culture lysates. The levels of muscle specific proteins were determined in triplicate treated and untreated cultures. These cultures were prepared and treated as above except that the plate size was increased to 150 mm and the muscle culture layer was scraped off for Western analysis as described in Sklar, R., and Brown, R. (*J. Neurol. Sci.* 101:73-81, 1991). The results presented in Table A indicate that rhGGF2 treatment increases the levels of several muscle specific proteins, including dystrophin, myosin heavy chain (MHC, adult slow and fast isoforms), but does not increase the levels of HSP72 or MHC neonate isoform to a similar level per amount of protein loaded on the Western. The levels of muscle specific proteins induced by rhGGF2 were similar to the quantitative increases in the number of myonuclei/mm^2 (Table 3).

TABLE 3

	Control ±SD	rhGGF2 Treatment ±SD	p value
Total Protein (μg)	554 ± 38.4	798 ± 73.6	0.007
Myonuclei/mm^2	29.0 ± 12.2	106 ± 24.1	0.008
MHC fast/μg protein	1.22 ± 0.47	4.00 ± 0.40	0.001
MHC slow/μg protein	0.17 ± 0.13	1.66 ± 0.27	0.001
MHC neonate/μg protein	0.30 ± 0.27	0.55 ± 0.04	0.199
dystrophin/μg protein	6.67 ± 0.37	25.5 ± 11.0	0.042
HSP 72/μg protein	3.30 ± 0.42	4.54 ± 0.08	0.008

The rhGGF2 dependent increase in the adult myosin heavy chain isoforms (slow is found in type I human muscle fibers; fast is found in type 2A and 2B human muscle fibers) may represent a maturation of the myotubes, as the neonatal isoform was not significantly increased by rhGGF2 treatment. During rat muscle development MHC isoforms switch from fetal to neonatal forms followed by a switch to mature adult slow and fast MHC isoforms (Periasamy et al. *J. Biol. Chem.* 259:13573-13578, 1984; Periasamy et al. *J. Biol. Chem.* 260:15856-15862, 1985; Wieczorek et al. *J. Cell Biol.* 101:618-629, 1985). While muscle can autonomously undergo some of these isoform transitions in the absence of neural cells or tissue, mouse muscle explants express the adult fast MHC isoform only when cultured in the presence of mouse spinal cord (Ecob-Prince et al. *J. Cell Biol.* 103:995-1005, 1986). Additional evidence that MHC isoform transitions are influenced by nerve was established by Whalen et al. (*Deve. Biol.* 141:24-40, 1990); after regeneration of notexin treated rat soleus muscles only

th adult fast MHC isoform was produced in th new denervated muscle, but innervated regenerated muscle made both fast and slow adult MHC isoforms. Thus the demonstration in Table 3 that rhGGF2 increases the synthesis of adult MHC isoforms indicates that rhGGF2 may induce a developmental maturation of muscle which may mimic neuronal innervation.

EXAMPLE 7

Neuregulins, including rhGGF2, induce the synthesis of acetylcholine receptors in muscle.

The expression of acetylcholine receptor (AchR) subunit proteins can be induced by exposing muscle cells to neuregulins. More specifically, we have shown that contacting muscle cells with rhGGF2 can induce the synthesis of AchR subunit proteins. This induction following rhGGF2 exposure was observed in two ways: first, we detected increased expression of human growth hormone via the product of a reporter gene construct and second we detected increased binding of alpha-bungarotoxin to cells.

In the following example a mouse myoblast cell line C2 was used. C2 cells were transfected with a transgene that contained the 5′ regulatory sequences of the AChR delta subunit gene of mouse linked to a human growth hormone full-length cDNA (Baldwin and Burden, 1988. J. Cell Biol. 107:2271-2279). This reporter construct allows the measurement of the induction of AChR delta gene expression by assaying the quantity of growth hormone secreted into the media. The line can be induced to form myotubes by lowering serum concentration in the media from 20% to 0.5%.

Specifically, mouse C2 myoblasts transfected with an AChR-human growth hormone reporter construct and were

assayed for expr ssi n f hGH foll wing treatment with rhGGF2. The results of two separate experiments are summarized in Table 4 and in Figures 6 (hGH expression) and 7 (hGH expression and alpha-bungarotoxin binding). Shown are the dose response curves for secreted human growth hormone and for bungarotoxin binding from muscle cultures treated with rhGGF2.

TABLE 4

Effects of rhGGF2 on the expression of AChR delta subunit/hGH transgene and the synthesis of AChR

	Exp 1	Exp 2	
GGF (ul)	hGH (ng/ml)	hGH (ng/ml)	AChR (cpm/mg protein)
0	9.3 + 2.1	5.7 + 2.1	822 + 170
0.1	-	6.8 + 1.5	891 + 134
0.5	-	12.0 + 0.9	993 + 35
1.0	-	9.7 + 2.3	818 + 67
5.0	17.5 + 2.8	14.7 + 3.5	1300 + 177
10.0	14.3 + 3.2	14.1 + 3.3	1388 + 137
15.0	22.0 + 1.4	-	-

C2 myotubes were treated with cold α-BTX (20 nM) for 1 hr. at 37°C, washed with culture medium twice and then treated with GGF2. Culture medium was adjusted with bovine serum albumin at the concentration of 1 mg/ml. 24 hours later, culture medium was removed and saved for hGH

assay. Muscle cultures ver treated with ^{125}I-α-BTX (20 nM) for 1 hour at 37°C, washed and scraped in PBS containing 1% SDS. Non-specific binding was determined in the presence of cold α-BTX (40 nM). The cell homogenate was counted for radioactivity and assayed for total protein amount.

The presence of rhGGF2 led to a greater than 2-fold increase in hGH gene expression, thereby indicating that rhGGF2 induced the synthesis of the delta subunit of the acetylcholine receptor. Furthermore, increased bungarotoxin binding is consistant with assembly of these subunit proteins into functional acetylcholine receptors. To strenthen the interpretation of these data the analysis was repeated on cultures that had the hGH reporter linked to a metallothiene promotor, which should not be responsive to rhGGF2. The results of that control experiment showed that the hGH response was mediated through transcriptional activation of the AchR delta subunit gene control elements.

These results indicate that rhGGF2 could be useful in replenishing AchRs as part of the therapy for the autoimmune disease Myasthenia gravis. This activity may also be beneficial in treatment of peripheral nerve regeneration and neuropathy by stimulating a key step in re-innervation of muscle.

EXAMPLE 8

Additional Mitogenic Activities of Purified GGF-I and GGF-II

The mitogenic activity of a highly purified sample containing both GGFs I and II was studied using a quantitative method, which allows a single microculture to be examined for DNA synthesis, cell morphology, cell number and expression of cell antigens. This technique

has been modified from a method previously reported by Muir et al., Analytical Biochemistry 185, 377-382, 1990. The main modifications are: 1) the use of uncoated microtiter plates, 2) the cell number per well, 3) the use of 5% Foetal Bovine Plasma (FBP) instead of 10% Foetal Calf Serum (FCS), and 4) the time of incubation in presence of mitogens and bromodeoxyuridine (BrdU), added simultaneously to the cultures. In addition the cell monolayer was not washed before fixation to avoid loss of cells, and the incubation time of monoclonal mouse anti-BrdU antibody and peroxidase conjugated goat anti-mouse immunoglobulin (IgG) antibody were doubled to increase the sensitivity of the assay. The assay, optimized for rat sciatic nerve Schwann cells, has also been used for several cell lines, after appropriate modifications to the cell culture conditions.

I. Methods of Mitogenesis Testing

On day 1, purified Schwann cells were plated onto uncoated 96 well plates in 5% FBP/Dulbecco's Modified Eagle Medium (DMEM) (5,000 cells/well). On day 2, GGFs or other test factors were added to the cultures, as well as BrdU at a final concentration of 10 μm. After 48 hours (day 4) BrdU incorporation was terminated by aspirating the medium and cells were fixed with 200 μl/well of 70% ethanol for 20 min at room temperature. Next, the cells were washed with water and the DNA denatured by incubation with 100 μl 2N HCl for 10 min at 37°C. Following aspiration, residual acid was neutralized by filling the wells with 0.1 M borate buffer, pH 9.0, and the cells were washed with phosphate buffered saline (PBS). Cells were then treated with 50 μl of blocking buffer (PBS containing 0.1% Triton X 100 and 2% normal goat serum) for 15 min at 37°C. After

aspirati n, m n cl nal m use anti-BrdU antibody (Dako Corp., Santa Barbara, CA) (50 µl/well, 1.4 µg/ml diluted in blocking buffer) was added and incubated for two hours at 37°C. Unbound antibodies were removed by three washes in PBS containing 0.1% Triton X-100 and peroxidase-conjugated goat anti-mouse IgG antibody (Dako Corp., Santa Barbara, CA) (50 µl/well, 2 µg/ml diluted in blocking buffer) was added and incubated for one hour at 37°C. After three washes in PBS/Triton and a final rinse in PBS, wells received 100 µl/well of 50 mM phosphate/citrate buffer, pH 5.0, containing 0.05% of the soluble chromogen o-phenylenediamine (OPD) and 0.02% H_2O_2. The reaction was terminated after 5-20 min at room temperature, by pipetting 80 µl from each well to a clean plate containing 40 µl/well of 2N sulfuric acid. The absorbance was recorded at 490nm using a plate reader (Dynatech Labs). The assay plates containing the cell monolayers were washed twice with PBS and immunocytochemically stained for BrdU-DNA by adding 100 µl/well of the substrate diaminobenzidine (DAB) and 0.02% H_2O_2 to generate an insoluble product. After 10-20 min the staining reaction was stopped by washing with water, and BrdU-positive nuclei observed and counted using an inverted microscope. occasionally, negative nuclei were counterstained with 0.001% Toluidine blue and counted as before.

II. Cell lines used for Mitogenesis Assays

Swiss 3T3 Fibroblasts: Cells, from Flow Labs, were maintained in DMEM supplemented with 10% FCS, penicillin and streptomycin, at 37°C in a humidified atmosphere of 10% CO_2 in air. Cells were fed or subcultured every two days. For mitogenic assay, cells were plated at a density of 5,000 cells/well in complete

medium and incubated for a week until cells were confluent and quiescent. The serum containing medium was removed and the cell monolayer washed twice with serum free-medium. 100 μl of serum free medium containing mitogens and 10μM of BrdU were added to each well and incubated for 48 hours. Dose responses to GGFs and serum or PDGF (as a positive control) were performed.

BHK (Baby Hamster Kidney) 21 C13 Fibroblasts: Cells from European Collection of Animal Cell Cultures (ECACC), were maintained in Glasgow Modified Eagle Medium (GMEM) supplemented with 5% tryptose phosphate broth, 5% FCS, penicillin and streptomycin, at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were fed or subcultured every two to three days. For mitogenic assay, cells were plated at a density of 2,000 cell/well in complete medium for 24 hours. The serum containing medium was then removed and after washing with serum free medium, replaced with 100 μl of 0.1% FCS containing GMEM or GMEM alone. GGFs and FCS or bFGF as positive controls were added, coincident with 10μM BrdU, and incubated for 48 hours. Cell cultures were then processed as described for Schwann cells.

C6 Rat Glioma Cell Line: Cells, obtained at passage 39, were maintained in DMEM containing 5% FCS, 5% Horse serum (HS), penicillin and streptomycin, at 37°C in a humidified atmosphere of 10% CO_2 in air. Cells were fed or subcultured every three days. For mitogenic assay, cells were plated at a density of 2,000 cells/well in complete medium and incubated for 24 hours. Then medium was replaced with a mixture of 1:1 DMEM and F12 medium containing 0.1% FCS, after washing in serum free medium. Dose responses to GGFs, FCS and αFGF were then

performed and cells were proc ssed through the ELISA as previously described for the other cell types.

PC12 (Rat Adrenal Pheochromocytoma Cells): Cells from ECACC, were maintained in RPMI 1640 supplemented with 10% HS, 5% FCS, penicillin and streptomycin, in collagen coated flasks, at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were fed every three days by replacing 80% of the medium. For mitogenic assay, cells were plated at a density of 3,000 cells/well in complete medium, on collagen coated plates (50 µl/well collagen, Vitrogen Collagen Corp., diluted 1 : 50, 30 min at 37°C) and incubated for 24 hours. The medium was then placed with fresh RPMI either alone or containing 1 mM insulin or 1% FCS. Dose responses to FCS/HS (1:2) as positive control and to GGFs were performed as before. After 48 hours cells were fixed and the ELISA performed as previously described.

<u>III. Results of Mitogenesis Assays</u>: All the experiments presented in this Example were performed using a highly purified sample from a Sepharose 12 chromatography purification step containing a mixture of GGF-I and GGF-II (GGFs).

First, the results obtained with the BrdU incorporation assay were compared with the classical mitogenic assay for Schwann cells based on [125]I-UdR incorporation into DNA of dividing cells, described by J. P. Brockes (*Methods Enzymol*. 147:217, 1987).

Fig. 12 shows the comparison of data obtained with the two assays, performed in the same cell culture conditions (5,000 cells/well, in 5% FBP/DMEM, incubated in presence of GGFs for 48 hrs). As clearly shown, the results are comparable, but BrdU incorporation assay

appears to be slightly more sensitive, as suggested by the shift of the curve to the left of the graph, i.e. to lower concentrations of GGFS.

As described under the section "Methods of Mitogenesis Testing", after the immunoreactive BrdU-DNA has been quantitated by reading the intensity of the soluble product of the OPD peroxidase reaction, the original assay plates containing cell monolayers can undergo the second reaction resulting in the insoluble DAB product, which stains the BrdU positive nuclei. The microcultures can then be examined under an inverted microscope, and cell morphology and the numbers of BrdU-positive and negative nuclei can be observed.

In Fig. 13A and Fig. 13B the BrdU-DNA immunoreactivity, evaluated by reading absorbance at 490 nm, is compared to the number of BrdU-positive nuclei and to the percentage of BrdU-positive nuclei on the total number of cells per well, counted in the same cultures. Standard deviations were less than 10%. The two evaluation methods show a very good correlation and the discrepancy between the values at the highest dose of GGFs can be explained by the different extent of DNA synthesis in cells detected as BrdU-positive.

The BrdU incorporation assay can therefore provide additional useful information about the biological activity of polypeptides on Schwann cells when compared to the (125) I-UdR incorporation assay. For example, the data reported in Fig. 15 show that GGFs can act on Schwann cells to induce DNA synthesis, but at lower doses to increase the number of negative cells present in the microculture after 48 hours.

The assay has then been used on several cell lines of different origin. In Fig. 15 the mitogenic responses of Schwann cells and Swiss 3T3 fibroblasts to GGFs are

compared; despite the weak response btain d in 3T3 fibroblasts, some clearly BrdU-positive nuclei were detected in these cultures. Control cultures were run in parallel in presence of several doses of FCS or human recombinant PDGF, showing that the cells could respond to appropriate stimuli (not shown).

The ability of fibroblasts to respond to GGFs was further investigated using the BHK 21 C13 cell line. These fibroblasts, derived from kidney, do not exhibit contact inhibition or reach a quiescent state when confluent. Therefore the experimental conditions were designed to have a very low background proliferation without compromising the cell viability. GGFs have a significant mitogenic activity on BHK21 C13 cells as shown by Fig. 16 and Fig. 17. Fig. 16 shows the Brdu incorporation into DNA by BHK 21 C13 cells stimulated by GGFS in the presence of 0.1% FCS. The good mitogenic response to FCS indicates that cell culture conditions were not limiting. In Fig. 17 the mitogenic effect of GGFs is expressed as the number of BrdU-positive and BrdU-negative cells and as the total number of cells counted per well. Data are representative of two experiments run in duplicates; at least three fields per well were counted. As observed for Schwann cells in addition to a proliferative effect at low doses, GGFs also increase the numbers of nonresponding cells surviving. The percentage of BrdU positive cells is proportional to the increasing amounts of GGFs added to the cultures. The total number of cells after 48 hours in presence of higher doses of GGFs is at least doubled, confirming that GGFs induce DNA synthesis and proliferation in BHK21 C13 cells. Under the same conditions, cells maintained for 48 hours in the presence

of 2% FCS showed an increase of about six fold (not shown).

C6 glioma cells have provided a useful model to study glial cell properties. The phenotype expressed seems to be dependent on the cell passage, the cells more closely resembling an astrocyte phenotype at an early stage, and an oligodendrocyte phenotype at later stages (beyond passage 70). C6 cells used in these experiments were from passage 39 to passage 52. C6 cells are a highly proliferating population, therefore the experimental conditions were optimized to have a very low background of BrdU incorporation. The presence of 0.1% serum was necessary to maintain cell viability without significantly affecting the mitogenic responses, as shown by the dose response to FCS (Fig. 18).

In Fig. 19 the mitogenic responses to aFGF (acidic Fibroblast growth factor) and GGFs are expressed as the percentages of maximal BrdU incorporation obtained in the presence of FCS (8%). Values are averages of two experiments, run in duplicates. The effect of GGFs was comparable to that of a pure preparation of aFGF. aFGF has been described as a specific growth factor for C6 cells (Lim R. et al., Cell Regulation 1:741-746, 1990) and for that reason it was used as a positive control. The direct counting of BrdU positive and negative cells was not possible because of the high cell density in the microcultures. In contrast to the cell lines so far reported, PC12 cells did not show any evident responsiveness to GGFS, when treated under culture conditions in which PC12 could respond to sera (mixture of FCS and HS as used routinely for cell maintenance). Nevertheless the number of cells plated per well seems to affect the behavior of PC12 cells, and therefore further experiments are required.

EXAMPLE 9

Amino acid sequences of purified GGF-I and GGF-II

Amino acid sequence analysis studies were performed using highly purified bovine pituitary GGF-I and GGF-II. The conventional single letter code was used to describe the sequences. Peptides were obtained by lysyl endopeptidase and protease V8 digests, carried out on reduced and carboxymethylated samples, with the lysyl endopeptidase digest of GGF-II carried out on material eluted from the 55-65 RD region of a 11% SDS-PAGE (MW relative to the above-quoted markers).

A total of 21 peptide sequences (see Fig. 8, SEQ ID Nos. 1-20, 165) were obtained for GGF-I, of which 12 peptides (see Fig. 9, SEQ ID Nos. 1, 22-29, 17, 19, and 32) are not present in current protein databases and therefore represent unique sequences. A total of 12 peptide sequences (see Fig. 10, SEQ ID Nos. 42-50 and 161-163) were obtained for GGF-II, of which 10 peptides (see Fig. 11, SEQ ID Nos. 42-50) are not present in current protein databases and therefore represent unique sequences (an exception is peptide GGF-II 06 which shows identical sequences in many proteins which are probably of no significance given the small number of residues). These novel sequences are extremely likely to correspond to portions of the true amino acid sequences of GGFs I and II.

Particular attention can be drawn to the sequences of GGF-I 07 and GGF-II 12, which are clearly highly related. The similarities indicate that the sequences of these peptides are almost certainly those of the assigned GGF species, and are most unlikely to be derived from contaminant proteins.

In addition, in peptide GGF-II 02, the sequence X S S is consistent with the presence of an N linked

carbohydrate moiety on an asparagine at the position denoted by X.

In general, in Figs. 8 and 10, X represents an unknown residue denoting a sequencing cycle where a single position could not be called with certainty either because there was more than one signal of equal size in the cycle or because no signal was present. As asterisk denotes those peptides where the last amino acid called corresponds to the last amino acid present in that peptide. In the remaining peptides, the signal strength after the last amino acid called was insufficient to continue sequence calling to the end of that peptide. The right hand column indicates the results of a computer database search using the GCG package FASTA and TFASTA programs to analyze the NBRF and EMBL sequence databases. The name of a protein in this column denotes identity of a portion of its sequence with the peptide amino acid sequence called allowing a maximum of two mismatches. A question mark denotes three mismatches allowed. The abbreviations used are as follows:

HMG-1	High Mobility Group protein-1
HMG-2	High Mobility Group protein-2
LH-alpha	Luteinizing hormone alpha subunit
LH-beta	Luteinizing hormone beta subunit

EXAMPLE 10

Isolating and Cloning of Nucleotide Sequences encoding proteins containing GGF-I and GGF-II peptides

Isolation and cloning of the GGF-II nucleotide sequences was performed as outlined herein, using peptide sequence information and library screening, and was performed as set out below. It will be appreciated that the peptides of Figs. 10 and 11 can be used as the

starting point for is lati n and cloning f GGF-I sequences by following the techniques described herein. Indeed, Fig. 20, SEQ ID Nos. 51-84) shows possible degenerate oligonucleotide probes for this purpose, and Fig. 22, SEQ ID Nos. 86-115, lists possible PCR primers. DNA sequence and polypeptide sequence should be obtainable by this means as with GGF-II, and also DNA constructs and expression vectors incorporating such DNA sequence, host cells genetically altered by incorporating such constructs/vectors, and protein obtainable by cultivating such host cells. The invention envisages such subject matter.

I. Design and Synthesis of oligonucleotide Probes and Primers

Degenerate DNA oligomer probes were designed by backtranslating the amino acid sequences (derived from the peptides generated from purified GGF protein) into nucleotide sequences. Oligomers represented either the coding strand or the non-coding strand of the DNA sequence. When serine, arginine or leucine were included in the oligomer design, then two separate syntheses were prepared to avoid ambiguities. For example, serine was encoded by either TCN or AGY as in 537 and 538 or 609 and 610. Similar codon splitting was done for arginine or leucine (e.g. 544, 545). DNA oligomers were synthesized on a Biosearch 8750 4-column DNA synthesizer using β-cyanoethyl chemistry operated at 0.2 micromole scale synthesis. Oligomers were cleaved off the column (500 angstrom CpG resins) and deprotected in concentrated ammonium hydroxide for 6-24 hours at 55-60°C. Deprotected oligomers were dried under vacuum (Speedvac) and purified by electrophoresis in gels of 15% acrylamide (20 mono : 1 bis), 50 mM Tris-borate-EDTA buffer

c ntaining 7M ur a. Full length oligomers w r d tected in the gels by UV shadowing, then the bands were excised and DNA oligomers eluted into 1.5 mls H20 for 4-16 hours with shaking. The eluate was dried, redissolved in 0.1 ml H_2O and absorbance measurements were taken at 260nm.

Concentrations were determined according to the following formula:

(A 260 x units/ml) (60.6/length = x μM)

All oligomers were adjusted to 50 μM concentration by addition of H_2O.

Degenerate probes designed as above are shown in Fig. 20, SEQ ID Nos. 54-88.

PCR primers were prepared by essentially the same procedures that were used for probes with the following modifications. Linkers of thirteen nucleotides containing restriction sites were included at the 5' ends of the degenerate oligomers for use in cloning into vectors. DNA synthesis was performed at 1 micromole scale using 1,000 angstrom CpG resins and inosine was used at positions where all four nucleotides were incorporated normally into degenerate probes. Purifications of PCR primers included an ethanol precipitation following the gel electrophoresis purification.

II. Library Construction and Screening

A bovine genomic DNA library was purchased from Stratagene (Catalogue Number: 945701). The library contained 2 x 10^6 15-20kb Sau3A1 partial bovine DNA fragments cloned into the vector lambda DashII. A bovine total brain cDNA library was purchased from Clonetech (Catalogue Number: BL 10139). Complementary DNA libraries were constructed (In Vitrogen; Stratagene) from mRNA prepared from bovine total brain, from bovine

pituitary and from bovine posterior pituitary. In Vitrogen prepared two cDNA libraries: one library was in the vector lambda g10, the other in vector pcDNAI (a plasmid library). The Stratagene libraries were prepared in the vector lambda unizap. Collectively, the cDNA libraries contained 14 million primary recombinant phage.

The bovine genomic library was plated on E. coli K12 host strain LE392 on 23 x 23 cm plates (Nunc) at 150,000 to 200,000 phage plaques per plate. Each plate represented approximately one bovine genome equivalent. Following an overnight incubation at 37°C, the plates were chilled and replicate filters were prepared according to procedures of Maniatis et al. (2:60-81). Four plaque lifts were prepared from each plate onto uncharged nylon membranes (Pall Biodyne A or MSI Nitropure). The DNA was immobilized onto the membranes by cross-linking under UV light for 5 minutes or, by baking at 80°C under vacuum for two hours. DNA probes were labelled using T4 polynucleotide kinase (New England Biolabs) with gamma 32P ATP (New England Nuclear; 6500 Ci/mmol) according to the specifications of the suppliers. Briefly, 50 pmols of degenerate DNA oligomer were incubated in the presence of 600 μCi gamma ^{32}P-ATP and 5 units T4 polynucleotide kinase for 30 minutes at 37°C. Reactions were terminated, gel electrophoresis loading buffer was added and then radiolabelled probes were purified by electrophoresis. 32P labelled probes were excised from gel slices and eluted into water. Alternatively, DNA probes were labelled via PCR amplification by incorporation of α-32P-dATP or α-32P dCTP according to the protocol of Schowalter and Sommer, Anal. Biochem 177:90-94 (1989). Probes labelled in PCR reactions were purified by desalting on Sephadex G-150 columns.

Prehybridization and hybridization were performed in GMC buffer (0.52 M NaPi, 7% SDS, 1% BSA, 1.5 mM EDTA, 0.1 M NaCl 10 mg/ml tRNA). Washing was performed in oligowash (160 ml 1 M Na_2HPO_4, 200 ml 20% SDS, 8.0 ml 0.5 M EDTA, 100 ml 5M NaCl, 3632 ml H20). Typically, 20 filters (400 sq. centimeters each) representing replicate copies of ten bovine genome equivalents were incubated in 200 ml hybridization solution with 100 pmols of degenerate oligonucleotide probe (128-512 fold degenerate). Hybridization was allowed to occur overnight at 5°C below the minimum melting temperature calculated for the degenerate probe. The calculation of minimum melting temperature assumes 2°C for an AT pair and 4°C for a GC pair.

Filters were washed in repeated changes of oligowash at the hybridization temperatures four to five hours and finally, in 3.2M tetramethylammonium chloride, 1% SDS twice for 30 min at a temperature dependent on the DNA probe length. For 20mers, the final wash temperature was 60°C. Filters were mounted, then exposed to X-ray film (Kodak XAR5) using intensifying screens (Dupont Cronex Lightening Plus). Usually, a three to five day film exposure at minus 80°C was sufficient to detect duplicate signals in these library screens. Following analysis of the results, filters could be stripped and reprobed. Filters were stripped by incubating through two successive cycles of fifteen minutes in a microwave oven at full power in a solution of 1% SDS containing 10mM EDTA pH8. Filters were taken through at least three to four cycles of stripping and reprobing with various probes.

III. Recombinant Phage Isolation, Growth and DNA Preparation

These procedures followed standard protocol as described in Recombinant DNA (Maniatis et al 2:60-2:81).

IV. Analysis of Isolated Clones Using DNA Digestion and Southern Blots

Recombinant Phage DNA samples (2 micrograms) were digested according to conditions recommended by the restriction endonuclease supplier (New England Biolabs). Following a four hour incubation at 37°C, the reactions products were precipitated in the presence of 0.1M sodium acetate and three volumes of ethanol. Precipitated DNA was collected by centrifugation, rinsed in 75% ethanol and dried. All resuspended samples were loaded onto agarose gels (typically 1% in TAE buffer; 0.04M Tris acetate, 0.002M EDTA). Gel runs were at 1 volt per centimeter from 4 to 20 hours. Markers included lambda Hind III DNA fragments and/or ØX174HaeIII DNA fragments (New England Biolabs). The gels were stained with 0.5 micrograms/ml of ethidium bromide and photographed. For southern blotting, DNA was first depurinated in the gel by treatment with 0.125 N HCl, denatured in 0.5 N NaOH and transferred in 20x SSC (3M sodium chloride, 0.03 M sodium citrate) to uncharged nylon membranes. Blotting was done for 6 hours up to 24 hours, then the filters were neutralized in 0.5 Tris HCl pH 7.5, 0.15 M sodium chloride, then rinsed briefly in 50 mM Tris-borate EDTA.

For cross-linking, the filters were wrapped first in transparent plastic wrap, then the DNA side exposed for five minutes to an ultraviolet light. Hybridization and washing was performed as described for library screening (see section 2 of this Example). For hybridization analysis to determine whether similar genes

exist in other species slight modifications were made. The DNA filter was purchased from Clonetech (Catalogue Number 7753-1) and contains 5 micrograms of EcoRI digested DNA from various species per lane. The probe was labelled by PCR amplification reactions as described in section 2 above, and hybridizations were done in 80% buffer B(2 g polyvinylpyrrolidine, 2 g Ficoll-400, 2 g bovine serum albumin, 50 ml 1M Tris-HCl (pH 7.5) 58 g NaCl, 1 g sodium pyrophosphate, 10 g sodium dodecyl sulfate, 950ml H_2O) containing 10% dextran sulfate. The probes were denatured by boiling for ten minutes then rapidly cooling in ice water. The probe was added to the hybridization buffer at 10^6 dpm ^{32}P per ml and incubated overnight at 60°C. The filters were washed at 60°C first in buffer B followed by 2X SSC, 0.1% SDS then in 1x SSC, 0.1% SDS. For high stringency, experiments, final washes were done in 0.1 x SSC, 1% SDS and the temperature raised to 65°C.

Southern blot data were used to prepare a restriction map of the genomic clone and to indicate which subfragments hybridized to the GGF probes (candidates for subcloning).

V. Subcloning of Segments of DNA Homologous to Hybridization Probes

DNA digests (e.g. 5 micrograms) were loaded onto 1% agarose gels then appropriate fragments excised from the gels following staining. The DNA was purified by adsorption onto glass beads followed by elution using the protocol described by the supplier (Bio 101). Recovered DNA fragments (100-200 ng) were ligated into linearized dephosphorylated vectors, e.g. pT3T7 (Ambion), which is a derivative of pUC18, using T4 ligase (New England Biolabs). This vector carries the E. coli β lactamase

g ne, henc , transf rmants can be selected on plates containing ampicillin. The vector also supplies β-galactosidase complementation to the host cell, therefore non-recombinants (blue) can be detected using isopropylthiogalactoside and Bluogal (Bethesda Research Labs). A portion of the ligation reactions was used to transform E. coli K12 XL1 blue competent cells (Stratagene Catalogue Number: 200236) and then the transformants were selected on LB plates containing 50 micrograms per ml ampicillin. White colonies were selected and plasmid mini preps were prepared for DNA digestion and for DNA sequence analysis. Selected clones were retested to determine if their insert DNA hybridized with the GGF probes.

VI. DNA Sequencing

Double stranded plasmid DNA templates were prepared from 5 ml cultures according to standard protocols. Sequencing was by the dideoxy chain termination method using Sequenase 2.0 and a dideoxynucleotide sequencing kit (US Biochemical) according to the manufacturers protocol (a modification of Sanger et al. PNAS; USA 74:5463 (1977)]. Alternatively, sequencing was done in a DNA thermal cycler (Perkin Elmer, model 4800) using a cycle sequencing kit (New England Biolabs; Bethesda Research Laboratories) and was performed according to manufacturers instructions using a 5'-end labelled primer. Sequence primers were either those supplied with the sequencing kits or were synthesized according to sequence determined from the clones. Sequencing reactions were loaded on and resolved on 0.4mm thick sequencing gels of 6% polyacrylamide. Gels were dried and exposed to X-Ray film. Typically, 35S was

incorporated when standard sequencing kits were used and a 32P end labelled primer was used for cycle sequencing reactions. Sequences were read into a DNA sequence editor from the bottom of the gel to the top (5' direction to 3') and data were analyzed using programs supplied by Genetics Computer Group (GCG, University of Wisconsin).

VII. RNA Preparation and PCR Amplification

Open reading frames detected in the genomic DNA and which contained sequence encoding GGF peptides were extended via PCR amplification of pituitary RNA. RNA was prepared from frozen bovine tissue (Pelfreeze) according to the guanidine neutral-CsCl procedure (Chirgwin et. al. Biochemistry 18:5294(1979).) Polyadenylated RNA was selected by oligo-dT cellulose column chromatography (Aviv and Leder PNAS (USA) 69:1408 (1972)).

Specific DNA target sequences were amplified beginning with either total RNA or polyadenylated RNA samples that had been converted to cDNA using the Perkin Elmer PCR/RNA Kit Number: N808-0017. First strand reverse transcription reactions used 1 μg template RNA and either primers of oligo dT with restriction enzyme recognition site linkers attached or specific antisense primers determined from cloned sequences with restriction sites attached. To produce the second strand, the primers either were plus strand unique sequences as used in 3' RACE reactions (Frohman et. al., PNAS (USA) 85:8998 (1988)) or were oligo dT primers with restriction sites attached if the second target site had been added by terminal transferase tailing first strand reaction products with dATP (e.g. 5' race reactions, Frohman et. al., ibid). Alternatively, as in anchored PCR reactions

the sec nd strand prim rs were deg nerat , hence, representing particular peptide sequences.

The amplification profiles followed the following general scheme: 1) five minutes soak file at 95°C; 2) thermal cycle file of 1 minute, 95°C; 1 minute ramped down to an annealing temperature of 45°C, 50°C or 55°C; maintain the annealing temperature for one minute; ramp up to 72°C over one minute; extend at 72°C for one minute or for one minute plus a 10 second auto extension; 3) extension cycle at 72°C, five minutes, and; 4) soak file 4°C for infinite time. Thermal cycle files (#2) usually were run for 30 cycles. A sixteen µl sample of each 100 µl amplification reaction was analyzed by electrophoresis in 2% Nusieve 1% agarose gels run in TAE buffer at 4 volts per centimeter for three hours. The gels were stained, then blotted to uncharged nylon membranes which were probed with labelled DNA probes that were internal to the primers.

Specific sets of DNA amplification products could be identified in the blotting experiments and their positions used as a guide to purification and reamplification. When appropriate, the remaining portions of selected samples were loaded onto preparative gels, then following electrophoresis four to five slices of 0.5 mm thickness (bracketing the expected position of the specific product) were taken from the gel. The agarose was crushed, then soaked in 0.5 ml of electrophoresis buffer from 2-16 hours at 40°C. The crushed agarose was centrifuged for two minutes and the aqueous phase was transferred to fresh tubes.

Reamplification was done on five microliters (roughly 1% of the product) of the eluted material using the same sets of primers and the reaction profiles as in the original reactions. When the reamplification

reactions were completed, samples were extracted with chloroform and transferred to fresh tubes. Concentrated restriction enzyme buffers and enzymes were added to the reactions in order to cleave at the restriction sites present in the linkers. The digested PCR products were purified by gel electrophoresis, then subcloned into vectors as described in the subcloning section above. DNA sequencing was done described as above.

VIII. DNA Sequence Analysis

DNA sequences were assembled using a fragment assembly program and the amino acid sequences deduced by the GCG programs GelAssemble, Map and Translate. The deduced protein sequences were used as a query sequence to search protein sequence databases using WordSearch. Analysis was done on a VAX Station 3100 workstation operating under VMS 5.1. The database search was done on SwissProt release number 21 using GCG Version 7.0.

IX. Results of Cloning and Sequencing of genes encoding GGF-I and GGF-II

As indicated above, to identify the DNA sequence encoding bovine GGF-II degenerate oligonucleotide probes were designed from GGF-II peptide sequences. GGF-II 12 (SEQ ID No. 44), a peptide generated via lysyl endopeptidase digestion of a purified GGF-II preparation (see Figs. 16 and 12) showed strong amino acid sequence homology with GGF-I 07 (SEQ ID No. 39), a tryptic peptide generated from a purified GGF-I preparation. GGF-II 12 was thus used to create ten degenerate oligonucleotide probes (see oligos 609, 610 and 649 to 656 in Fig. 20, SEQ ID Nos. 66, 67, 68 and 75, respectively). A duplicate set of filters were probed with two sets (set 1=609, 610; set 2=649-5656) of probes encoding two

overlapping portions of GGF-II 12. Hybridization signals were observed, but, only one clone hybridized to both probe sets. The clone (designated GGF2BG1) was purified.

Southern blot analysis of DNA from the phage clone GGF2BG1 confirmed that both sets of probes hybridized with that bovine DNA sequence, and showed further that both probes reacted with the same set of DNA fragments within the clone. Based on those experiments a 4 kb Eco RI sub-fragment of the original clone was identified, subcloned and partially sequenced. Fig. 21 shows the nucleotide sequence, SEQ ID No. 89) and the deduced amino acid sequence of the initial DNA sequence readings that included the hybridization sites of probes 609 and 650, and confirmed that a portion of this bovine genomic DNA encoded peptide 12 (KASLADSGEYM).

Further sequence analysis demonstrated that GGF-II 12 resided on a 66 amino acid open reading frame (see below) which has become the starting point for the isolation of overlapping sequences representing a putative bovine GGF-II gene and a cDNA.

Several PCR procedures were used to obtain additional coding sequences for the putative bovine GGF-II gene. Total RNA and oligo dT-selected (poly A containing) RNA samples were prepared from bovine total pituitary, anterior pituitary, posterior pituitary, and hypothalamus. Using primers from the list shown in Fig. 22, SEQ ID Nos. 109-119, one-sided PCR reactions (RACE) were used to amplify cDNA ends in both the 3' and 5' directions, and anchored PCR reactions were performed with degenerate oligonucleotide primers representing additional GGF-II peptides. Fig. 29 summarizes the contiguous DNA structures and sequences obtained in those experiments. From the 3' RACE reactions, three alternatively spliced cDNA sequences were produced,

which have been cloned and s quenc d. A 5' RACE reaction led to the discovery of an additional exon containing coding sequence for at least 52 amino acids. Analysis of that deduced amino acid sequence revealed peptides GGF-II-6 and a sequence similar to GGF-I-18 (see below). The anchored PCR reactions led to the identification of (cDNA) coding sequences of peptides GGF-II-1, 2, 3 and 10 contained within an additional cDNA segment of 300 bp. The 5' limit of this segment (i.e., segment E, see Fig. 30) is defined by the oligonucleotide which encodes peptide GGF-II-1 and which was used in the PCR reaction (additional 5' sequence data exists as described for the human clone in Example 11). Thus this clone contains nucleotide sequences encoding six out of the existing total of nine novel GGF-II peptide sequences.

The cloned gene was characterized first by constructing a physical map of GGF2BG1 that allowed us to position the coding sequences as they were found (see below, Fig. 30). DNA probes from the coding sequences described above have been used to identify further DNA fragments containing the exons on this phage clone and to identify clones that overlap in both directions. The putative bovine GGF-II gene is divided into at least 5 coding segments. Coding segments are defined as discrete lengths of DNA sequence which can be translated into polypeptide sequences using the universal genetic code. The coding segments described in Fig. 36 and referred to in the present application are: 1) particular exons present within the GGF gene (e.g. coding segment a), or 2) derived from sets of two or more exons that appear in specific sub-groups of mRNAs, where each set can be translated into the specific polypeptide segments as in the gene products shown. The polypeptide segments

r f rred to in th claims are the translation products of the analogous DNA coding segments. Only coding segments A and B have been defined as exons and sequenced and mapped thus far. The summary of the contiguous coding sequences identified is shown in Fig. 31. The exons are listed (alphabetically) in the order of their discovery. It is apparent from the intron/exon boundaries that exon B may be included in cDNAs that connect coding segment E and coding segment A. That is, exon B cannot be spliced out without compromising the reading frame. Therefore, we suggest that three alternative splicing patterns can produce putative bovine GGF-II cDNA sequences 1, 2 and 3. The coding sequences of these, designated GGF2BPP1.CDS, GGF2BPP2.CDS and GGF2BPP3.CDS, respectively, are given in Figs. 27A (SEQ ID No. 129), 27B (SEQ ID No. 130), and 27C (SEQ ID No. 131), respectively. The deduced amino acid sequence of the three cDNAs is also given in Figs. 27A, (SEQ ID No. 129), 27B (SEQ ID No. 130), and 27C (SEQ ID No. 131).

The three deduced structures encode proteins of lengths 206, 281 and 257 amino acids. The first 183 residues of the deduced protein sequence are identical in all three gene products. At position 184 the clones differ significantly. A codon for glycine GGT in GGF2BPP1 also serves as a splice donor for GGF2BPP2 and GGF2BPP3, which alternatively add on exons C, C/D, C/D' and D or C, C/D and D, respectively, and shown in Fig. 32, SEQ ID No. 145). GGFIIBPP1 is a truncated gene product which is generated by reading past the coding segment A splice junction into the following intervening sequence (intron). This represents coding segment A' in Fig. 30 (SEQ ID No. 136). The transcript ends adjacent to a canonical AATAAA polyadenylation sequence, and we suggest that this truncated gene product represents a

bona fide mature transcript. The other two longer gene products share the same 3′ untranslated sequence and polyadenylation site.

All three of these molecules contain six of the nine novel GGF-II peptide sequences (see Fig. 11) and another peptide is highly homologous to GGF-I-18 (see Fig. 26). This finding gives a high probability that this recombinant molecule encodes at least a portion of bovine GGF-II. Furthermore, the calculated isoelectric points for the three peptides are consistent with the physical properties of GGF-I and II. Since the molecular size of GGF-II is roughly 60 kD, the longest of the three cDNAs should encode a protein with nearly one-half of the predicted number of amino acids.

A probe encompassing the B and A exons was labelled via PCR amplification and used to screen a cDNA library made from RNA isolated from bovine posterior pituitary. One clone (GGF2BPP5) showed the pattern indicated in Fig. 29 and contained an additional DNA coding segment (G) between coding segments A and C. The entire nucleic acid sequence is shown in Fig. 31 (SEQ ID No. 144). The predicted translation product from the longest open reading frame is 241 amino acids. A portion of a second cDNA (GGF2BPP4) was also isolated from the bovine posterior pituitary library using the probe described above. This clone showed the pattern indicated in Fig. 29. This clone is incomplete at the 5′ end, but is a splicing variant in the sense that it lacks coding segments G and D. BPP4 also displays a novel 3′ end with regions H, K and L beyond region C/D. The sequence of BPP4 is shown in Fig. 33 (SEQ ID No. 146).

EXAMPLE 11

GGF Sequences in Various Species

The GGF proteins are th members of a new superfamily of proteins. In high stringency cross hybridization studies (DNA blotting experiments) with other mammalian DNAs we have shown, clearly, that DNA probes from this bovine recombinant molecule can readily detect specific sequences in a variety of samples tested. A highly homologous sequence is also detected in human genomic DNA. The autoradiogram is shown in Fig. 28. The signals in the lanes containing rat and human DNA represent the rat and human equivalents of the GGF gene, the sequences of several cDNA's encoded by this gene have been recently reported by Holmes et al. (Science 256: 1205 (1992)) and Wen et al. (Cell 69: 559 (1992)).

EXAMPLE 12

Isolation of a Human Sequence Encoding Human GGF2

Several human clones containing sequences from the bovine GGFII coding segment E were isolated by screening a human cDNA library prepared from brain stem (Stratagene catalog #935206). This strategy was pursued based on the strong link between most of the GGF2 peptides (unique to GGF2) and the predicted peptide sequence from clones containing the bovine E segment. This library was screened as described in Example 8, Section II using the oligonucleotide probes 914-919 listed below.

914TCGGGCTCCATGAAGAAGATGTA	(SEQ ID NO: 179)
915TCCATGAAGAAGATGTACCTGCT	(SEQ ID NO: 180)
916ATGTACCTGCTGTCCTCCTTGA	(SEQ ID NO: 181)
917TTGAAGAAGGACTCGCTGCTCA	(SEQ ID NO: 182)
918AAAGCCGGGGGCTTGAAGAA	(SEQ ID NO: 183)
919ATGARGTGTGGGCGGCGAAA	(SEQ ID NO: 184)

Clones detected with these probes were further analyzed by hybridization. A probe derived from coding segment A (see Fig. 30), which was produced by labeling a

polymerase chain reaction (PCR) product from s gment A, was also used to screen the primary library. Several clones that hybridized with both A and E derived probes were selected and one particular clone, GGF2HBS5, was selected for further analysis. This clone is represented by the pattern of coding segments (EBACC/D'D as shown in Fig. 30). The E segment in this clone is the human equivalent of the truncated bovine version of E shown in Fig. 30. GGF2HBS5 is the most likely candidate to encode GGF-II of all the "putative" GGF-II candidates described. The length of coding sequence segment E is 786 nucleotides plus 264 bases of untranslated sequence. The predicted size of the protein encoded by GGF2HBS5 is approximately 423 amino acids (approximately 45 kilodaltons, see Fig. 44, SEQ ID NO: 21), which is similar to the size of the deglycosylated form of GGF-II (see Example 20). Additionally, seven of the GGF-II peptides listed in Fig. 26 have equivalent sequences which fall within the protein sequence predicted from region E. Peptides II-6 and II-12 are exceptions, which fall in coding segment B and coding segment A, respectively. RNA encoding the GGF2HBS5 protein was produced in an in vitro transcription system driven by the bacteriophage T7 promoter resident in the vector (Bluescript SK [Stratagene Inc.] see Fig. 47) containing the GGF2HBS5 insert. This RNA was translated in a cell free (rabbit reticulocyte) translation system and the size of the protein product was 45 Kd. Additionally, the cell-free product has been assayed in a Schwann cell mitogenic assay to confirm biological activity. Schwann cells treated with conditioned medium show both increased proliferation as measured by incorporation of ^{125}I-Uridine and phosphorylation on tyrosine of a protein in the 185 kilodalton range.

Thus the size f the product encod d by GGF2HBS5 and the presence of DNA sequences which encode human peptides highly homologous to the bovine peptides shown in Fig. 11 confirm that GGF2HBS5 encodes the human equivalent of bovine GGF2. The fact that conditioned media prepared from cells transformed with this clone elicits Schwann cell mitogenic activity confirms that the GGFIIHBS5 gene produce (unlike the BPP5 gene product) is secreted. Additionally the GGFIIBPP5 gene product seems to mediate the Schwann cell proliferation response via a receptor tyrosine kinase such as $p185^{erbB2}$ or a closely related receptor (see Example 19).

EXAMPLE 13

Expression of Human Recombinant GGF2 in Mammalian and Insect Cells

The GGF2HBS5 cDNA clone encoding human GGF2 (as described in Example 12 and also referred to herein as HBS5) was cloned into vector pcDL-SRα296 and COS-7 cells were transfected in 100 mm dishes by the DEAE-dextran method. Cell lysates or conditioned media from transiently expressing COS cells were harvested at 3 or 4 days post-transfection. To prepare lysates, cell monolayers were washed with PBS, scraped from the dishes lysed by three freeze/thaw cycles in 150 µm of 0.25 M Tris-HCl, pH8. Cell debris was pelleted and the supernatant recovered. Conditioned media samples (7 mls.) were collected, then concentrated and buffer exchanged with 10 mm Tris, pH 7.4 using Centiprep-10 and Centricon-10 units as described by the manufactures (Amicon, Beverly, MA). Rat nerve Schwann cells were assayed for incorporation of DNA synthesis precursors, as described. Conditioned media or cell lysate samples were tested in the Schwann cell proliferation assay as described in Marchionni et al., *Nature* 362:313 (1993).

The cDNA, GGF2HBS5, encoding GGF2 directed the secretion of the protein product to the medium. Minimal activity was detectable inside the cells as determined by assays using cell lysates. GGF2HFB1 and GGFBPP5 cDNA's failed to direct the secretion of the product to the extracellular medium. GGF activity from these clones was detectable only in cell lysates.

Recombinant GGF2 was also expressed in CHO cells. The GGF2HBS5 cDNA encoding GGF2 was cloned into the EcoRI site of vector pcdhfrpolyA and transfected into the DHFR negative CHO cell line (GG44) by the calcium phosphate coprecipitation method. Clones were selected in nucleotide and nucleoside free α medium (Gibco) in 96-well plates. After 3 weeks, conditioned media samples from individual clones were screened for expression of GGF by the Schwann cell proliferation assay as described in Marchionni et al., Nature 362:313 (1993). Stable clones which secreted significant levels of GGF activity into the medium were identified. Schwann cell proliferation activity data from different volume aliquots of CHO cell conditioned medium were used to produce the dose response curve shown in Fig. 46 (Graham and Van Der Eb, Virology 52:456, 1973). This material was analyzed on a Western blot probed with polyclonal antisera raised against a GGF2 specific peptide. A band of approximately 65 Kd (the expected size of GGF2 extracted from pituitary) is specifically labeled (Fig. 48, lane 12).

Recombinant GGF2 was also expressed in insect cells using the Baculovirus expression. Sf9 insect cells were infected with baculovirus containing the GGF2HBS5 cDNA clone at a multiplicity of 3-5 (10^6 cells/ml) and cultured in Sf900-II medium. Schwann cell mitogenic activity was secreted into the extracellular medium.

Different volumes of insect cell conditioned medium were tested in the Schwann cell proliferation assay in the absence of forskolin and the data used to produce a dose response curve.

This material was also analyzed on a Western blot (Fig. 45B) probed with the GGF II specific antibody described above.

The methods used in this example were as follows:

Schwann cell mitogenic activity of recombinant human and bovine glial growth factors was determined as follows: Mitogenic responses of cultured Schwann cells were measured in the presence of 5 μM forskolin using crude recombinant GGF preparations obtained from transient mammalian expression experiments. Incorporation of [^{125}I]-Urd was determined following an 18-24 hour exposure to materials obtained from transfected or mock transfected cos cells as described in the Methods. The mean and standard deviation of four sets of data are shown. The mitogenic response to partially purified native bovine pituitary GGF (carboxymethyl cellulose fraction; Goodearl et al., submitted) is shown (GGF) as a standard of one hundred percent activity.

cDNAs (Fig. 46, SEQ ID NOs. 166-168) were cloned into pcDL-SRα296 (Takebe et al., Mol. Cell Biol. 8:466-472 (1988)), and COS-7 cells were transfected in 100 mm dishes by the DEAE-dextran method (Sambrook et al., In *Molecular Cloning. A Laboratory Manual, 2nd. ed.* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989)). Cell lysates or conditioned media were harvested at 3 or 4 days post-transfection. To prepare lysates, cell monolayers were washed with PBS, scraped from the dishes, and lysed by three freeze/than cycles in 150 μl of 0.25 M Tris-HCl, pH 8. Cell debris was pelleted and

the supernate recovered. Conditioned media samples (7 mls) were collected, then concentrated and buffer exchanged with 10 mM Tris, pH 7.4 using Centriprep-10 and Centricon-10 units are described by the manufacturers (Amicon, Beverly, MA). Rat sciatic nerve Schwann cells were assayed for incorporation of DNA synthesis precursors, as described (Davis and Stroobant, J. Cell Biol. 110:1353-1360 (1990); Brockes et al., Brain Res. 165:105-118 (1979)).

Western blot of recombinant CHO cell conditioned medium were performed as follows: A recombinant CHO clone was cultured in MCDB302 protein-free for 3 days. 2 ml of conditioned medium was harvested, concentrated, buffered exchanged against 10 mM Tris-HCl, pH 7.4 and lyophilized to dryness. The pellet was resuspended in SDS-PAGE sample buffer, subjected to reducing SDS gel electrophoresis and analyzed by Western blotting with a GGF peptide antibody. A CHO control was done by using conditioned medium from untransfected CHO-DG44 host and the CHO HBS5 levels were assayed using conditioned medium from a recombinant clone.

EXAMPLE 14

Identification of Functional Elements of GGF

The deduced structures of the family of GGF sequences indicate that the longest forms (as represented by GGF2BPP4) encode transmembrane proteins where the extracellular part contains a domain which resembles epidermal growth factor (see Carpenter and Wahl in Peptide Growth Factors and Their Receptors I pp. 69-133, Springer-Verlag, NY 1991). The positions of the cysteine residues in coding segments C and C/D or C/D' peptide sequence are conserved with respect to the analogous residues in the epidermal growth factor (EGF) peptide

sequenc (s e Fig. 32, SEQ ID Nos. 147-149). This suggests that the extracellular domain functions as receptor recognition and biological activation sites. Several of the variant forms lack the H, K, and L coding segments and thus may be expressed as secreted, diffusible biologically active proteins. GGF DNA sequences encoding polypeptides which encompass the EGF-like domain (EGFL) can have full biological activity for stimulating glial cell mitogenic activity.

Membrane bound versions of this protein may induce Schwann cell proliferation if expressed on the surface of neurons during embryogenesis or during nerve regeneration (where the surfaces of neurons are intimately associated with the surfaces of proliferating Schwann cells).

Secreted (non membrane bound) GGFs may act as classically diffusible factors which can interact with Schwann cells at some distance from their point of secretion. Other forms may be released from intracells by sources via tissue injury and cell disruption. An example of a secreted GGF is the protein encoded by GGF2HBS5; this is the only GGF known which has been found to be directed to the exterior of the cell. Secretion is probably mediated via an N-terminal hydrophobic sequence found only in region E, which is the N-terminal domain contained within recombinant GGF2 encoded by GGF2HBS5.

Other GGF's appear to be non-secreted. These GGFs may be injury response forms which are released as a consequence of tissue damage.

Other regions of the predicted protein structure of GGF2 (encoded by GGF2HBS5) and other proteins containing regions B and A exhibit similarities to the human basement membrane heparan sulfate proteoglycan core protein. The peptide ADSGEY, which is located next to the second cysteine of the C2 immunoglobulin fold in

these GGF's, occurs in nine of twenty-two C-2 repeats found in that basal lamina protein. This evidence strongly suggests that these proteins may associate with matrix proteins such as those associated with neurons and glia, and may suggest a method for sequestration of glial growth factors at target sites.

EXAMPLE 15

Purification of GGFs from Recombinant Cells

In order to obtain full length or portions of GGFs to assay for biological activity, the proteins can be overproduced using cloned DNA. Several approaches can be used. A recombinant E. coli cell containing the sequences described above can be constructed. Expression systems such as pNH8a or pHH16a (Stratagene, Inc.) can be used for this purpose by following manufacturers procedures. Alternatively, these sequences can be inserted in a mammalian expression vector and an overproducing cell line can be constructed. As an example, for this purpose DNA encoding a GGF, clone GGF2BPP5 has been expressed in COS cells and can be expressed in Chinese hamster ovary cells using the pMSXND expression vector (Lee and Nathans, J. Biol. Chem. 263, 3521-3527, (1981)). This vector containing GGF DNA sequences can be transfected into host cells using established procedures.

Transient expression can be examined or G418-resistant clones can be grown in the presence of methotrexate to select for cells that amplify the dhfr gene (contained on the pMSXND vector) and, in the process, co-amplify the adjacent GGF protein encoding sequence. Because CHO cells can be maintained in a totally protein-free medium (Hamilton and Ham, In Vitro 13, 537-547 (1977)), the desired protein can be purified

from the medium. Western analysis using the antisera produced in Example 17 can be used to detect the presence of the desired protein in the conditioned medium of the overproducing cells.

The desired protein (rGGF2) was purified from the medium conditioned by transiently expressing cos cells as follows. rGGF II was harvested from the conditioned medium and partially purified using Cation Exchange Chromatography (POROS-HS). The column was equilibrated with 33.3 mM MES pH 6.0. Conditioned media was loaded at flow rate of 10 ml/min. The peak containing Schwann cell proliferation activity and immunoreactive (using the polyclonal antisera was against a GGF2 peptide described above) was eluted with 50 mM Tris, 1M NaCl pH 8.0.

rhGGF2 is also expressed using a stable Chinese Ovary Hamster cell line. rGGF2 from the harvested conditioned media was partially purified using Cation Exchange Chromatograph (POROS-HS). The column was equilibrated with PBS pH 7.4. Conditioned media was loaded at 10 ml/min. The peak containing the Schwann Cell Proliferative activity and immunoreactivity (using GGF2 polyclonal antisera) was eluted with 50 mM Hepes, 500 mM NaCl pH 8.0. An additional peak was observed at 50 mM Hepes, 1M NaCl pH 8.0 with both proliferation as well as immunoreactivity (Fig. 45).

rhGGF2 can be further purified using Hydrophobic Interaction Chromatography as a high resolution step; Cation exchange/Reserve phase Chromatography (if needed as second high resolution step); A viral inactivation step and a DNA removal step such as Anion exchange chromatography.

Schwann Cell Proliferation Activity of recombinant GGF2 peak eluted from the Cation Exchange column was determined as follows: Mitogenic responses of the

cultured Schwann cells were measured in the presence of 5 M Forskolin using the peak eluted by 50 mM Tris 1 M NaCl pH 8.0. The peak was added at 20 1, 10 1 (1:10) 10 1 and (1:100) 10 1. Incorporation of ^{125}I-Uridine was determined and expressed as (CPM) following an 18-24 hour exposure.

An immunoblot using polyclonal antibody raised against a peptide of GGF2 was carried out as follows: 10 1 of different fractions were ran on 4-12% gradient gels. The gels were transferred on to Nitrocellulose paper, and the nitrocellulose blots were blocked with 5% BSA and probed with GGF2-specific antibody (1:250 dilution). ^{125}I protein A (1:500 dilution, Specific Activity = 9.0/Ci/g) was used as the secondary antibody. The immunoblots were exposed to Kodax X-Ray films for 6 hours. The peak fractions eluted with 1 M NaCl showed an immunoreactive band at 69K.

GGF2 purification on cation exchange columns was performed as follows: CHO cell conditioned media expressing rGGFII was loaded on the cation exchange column at 10 ml/min. The column was equilibrated with PBS pH 7.4. The elution was achieved with 50 mM Hepes 500 mM NaCl pH 8.0 and 50 mM Hepes 1M NaCl pH 8.0 respectively. All fractions were analyzed using the Schwann cell proliferation assay (CPM) described herein. The protein concentration (mg/ml) was determined by the Bradford assay using BSA as the standard.

A Western blot using 10 1 of each fraction was performed and immunoreactivity and the Schwann cell activity were observed to co-migrate.

The protein may be assayed at various points in the procedure using a Western blot assay. Alternatively, the Schwann cell mitogenic assay described herein may be used to assay the expressed product of the full length

cl n or any bi logically active p rtions ther of. The full length clone GGF2BPP5 has been expressed transiently in COS cells. Intracellular extracts of transfected COS cells show biological activity when assayed in the Schwann cell proliferation assay described in Example 8. In addition, the full length close encoding GGF2HBS5 has been expressed transiently in COS cells. In this case both cell extract and conditioned media show biological activity in the Schwann cell proliferation assay described in Example 8. Any member of the family of splicing variant complementary DNA's derived from the GGF gene (including the Heregulins) can be expressed in this manner and assayed in the Schwann cell proliferation assay by one skilled in the art.

Alternatively, recombinant material may be isolated from other variants according to Wen et al. (Cell 69:559 (1992)) who expressed the splicing variant Neu differentiation factor (NDF) in COS-7 cells. cDNA clones inserted in the pJT-2 eukaryotic plasmid vector are under the control of the SV40 early promoter, and are 3'-flanked with the SV40 termination and polyadenylation signals. COS-7 cells were transfected with the pJT-2 plasmid DNA by electroporation as follows: 6×10^6 cells (in 0.8 ml of DMEM and 10% FEBS) were transferred to a 0.4 cm cuvette and mixed with 20 μg of plasmid DNA in 10 μl of TE solution (10 mM Tris-HCl (pH 8.0), 1 mM EDTA). Electroporation was performed at room temperature at 1600 V and 25 μF using a Bio-Rad Gene Pulser apparatus with the pulse controller unit set at 200 ohms. The cells were then diluted into 20 ml of DMEM, 10% FBS and transferred into a T75 flask (Falcon). After 14 hr. of incubation at 37°C, the medium was replaced with DMEM, 1% FBS, and the incubation continued for an additional 48 hr. Conditioned medium containing recombinant protein

which was harvested from the cells demonstrated biological activity in a cell line expressing the receptor for this protein. This cell line (cultured human breast carcinoma cell line AU 565) was treated with recombinant material. The treated cells exhibited a morphology change which is characteristic of the activation of the erbB2 receptor. Conditioned medium of this type also can be tested in the Schwann cell proliferation assay.

EXAMPLE 16

N-terminal sequence analysis

The cDNA encoding hGGF2 was cloned into the amplifiable vector pcdhfrpolyA and transfected into CHO-DG44 cells for stable expression. rhGGF2 is secreted into the conditioned media. The ability of the recombinant GGF2 to be secreted is presumably mediated through the N-terminal hydrophobic stretch (signal sequence). A signal sequence, once having initiated export of a growing protein chain across the rough endoplasmic reticulum, is cleaved from the mature protein at a specific site. N-terminal sequence analysis of the expressed and purified rhGGF2 indicates the site of cleavage as shown below. The sequence of the first 50 amino acid residues at the N-terminus of the protein was

c nfirmed by N-terminal sequence analysis (Table 5), below.

TABLE 5

N-terminal sequence analysis of rhGGF2

Cycle #	Primary Sequence	pMoles
1	Gly (G)	210.6
2	Asn (N)	163
3	GLU (E)	149
4	Ala (A)	220
5	Ala (A)	180
6	Pro (P)	173
7	Ala (A)	177
8	Gly (G)	154.9
9	Ala (A)	162.4
10	Ser (S)	65.4
11	Val (V)	132.7
12	Val (V) *(Cys)	11.7
13	Tyr (Y)	112.7
14	Ser (S)	47.6
15	Ser (S)	27.1

The N-terminal sequence analysis is performed by Edman Degradation Process
*Cys residues are destroyed by the Edman Degradation Process and cannot be detected

The following sequence (SEQ ID NO: 185) represents the amino acid sequence of hGGF2. The shaded area indicates the cleaved signal sequence.

[illegible]
GNEAAPAGAS VCYSSPPSVG SVQELAQRAA VVIEGKVHPQ RRQQGALDRK
AAAAAGEAGA WGGDREPPAA GPRALGPPAE EPLLAANGTV PSWPTAPVPS

AGEPGEEAPY LVKVHQVWAV KAGGLKKDSL LTVRLGTWGH PAFPSCGRLK EDSRYIFFME PDANSTSRAP AAFRASFPPL ETGRNLKKEV SRVLCKRCAL PPQLKEMKSQ ESAAGSKLVL RCETSSEYSS LRFKWFKNGN ELNRKNKPQN IKIQKKPGKS ELRINKASLA DSGEYMCKVI SKLGNDSASA NITIVESNAT STSTTGTSHL VKCAEKEKTF CVNGGECFMV KDLSNPSRYL CKCPNEFTGD RCQNYVMASF YSTSTPFLSL PE (SEQ ID NO: 185)

The shaded area represents experimentally determined 15 amino acid residues at the N-terminal of the rhGGF2, indicating A_{50}-G_{51} bond to be the cleavage site for the signal sequence.

EXAMPLE 17

Isolation of a Further Splicing Variant

Methods for updating other neuregulins descsribed in U.S. patent application Serial No. 07/965,173, filed October 23, 1992, incorporated herein by reference, produced four closely related sequences (heregulin α, β1, β2, β3) which arise as a result of splicing variation. Peles et al. (Cell 69:205 (1992)), and Wen et al. (Cell 69:559 (1992)) have isolated another splicing variant (from rat) using a similar purification and cloning approach to that described in Examples 1-9 and 11 involving a protein which binds to $p185^{erbB2}$. The cDNA clone was obtained as follows (via the purification and sequencing of a $p185^{erbB2}$ binding protein from a transformed rat fibroblast cell line).

A $p185^{erbB2}$ binding protein was purified from conditioned medium as follows. Pooled conditioned medium from three harvests of 500 roller bottles (120 liters total) was cleared by filtration through 0.2 μ filters and concentrated 31-fold with a Pelicon ultrafiltration system using membranes with a 20kd molecular size cutoff. All the purification steps were performed by using a

Pharmacia fast pr t in liquid chromatography system. The concentrated material was directly loaded on a column of heparin-Sepharose (150 ml, preequilibrated with phosphate-buffered saline (PBS)). The column was washed with PBS containing 0.2 M NaCl until no absorbance at 280 nm wavelength could be detected. Bound proteins were then eluted with a continuous gradient (250 ml) of NaCl (from 0.2 M to 1.0 M), and 5 ml fractions were collected. Samples (0.01 ml of the collected fractions were used for the quantitative assay of the kinase stimulatory activity. Active fractions from three column runs (total volume = 360 ml) were pooled, concentrated to 25 ml by using a YM10 ultrafiltration membrane (Amicon, Danvers, MA), and ammonium sulfate was added to reach a concentration of 1.7 M. After clearance by centrifugation (10,000 x g, 15 min.), the pooled material was loaded on a phenyl-Superose column (HR10/10, Pharmacia). The column was developed with a 45 ml gradient of $(NH_4)_2SO_4$ (from 1.7 M to no salt) in 0.1 M Na_2PO_4 (pH 7.4), and 2 ml fractions were collected and assayed (0.002 ml per sample) for kinase stimulation (as described in Example 19). The major peak of activity was pooled and dialyzed against 50 mM sodium phosphate buffer (pH 7.3). A Mono-S cation-exchange column (HR5/5, Pharmacia) was preequilibrated with 50 mM sodium phosphate. After loading the active material (0.884 mg of protein; 35 ml), the column was washed with the starting buffer and then developed at a rate of 1 ml/min. with a gradient of NaCl. The kinase stimulatory activity was recovered at 0.45-0.55 M salt and was spread over four fractions of 2 ml each. These were pooled and loaded directly on a Cu^{+2} chelating columns (1.6 ml, HR2/5 chelating Superose, Pharmacia). Most of the proteins adsorbed to the resin, but they gradually eluted with a

30 ml lin ar gradient f ammonium chloride (0-1 M). The activity eluted in a single peak of protein at the range of 0.05 to 0.2 M NH_4Cl. Samples from various steps of purification were analyzed by gel electrophoresis followed by silver staining using a kit from ICN (Costa Mesa, CA), and their protein contents were determined with a Coomassie blue dye binding assay using a kit from Bio-Rad (Richmond, CA).

The p44 protein (10 μg) was reconstituted in 200 μl of 0.1 M ammonium bicarbonate buffer (pH 7.8). Digestion was conducted with L-1-tosyl-amide 2-phenylethyl chloromethyl ketone-treated trypsin (Serva) at 37°C for 18 hr. at an enzyme-to-substrate ratio of 1:10. The resulting peptide mixture was separated by reverse-phase HPLC and monitored at 215 nm using a Vydac C4 micro column (2.1 mm i.d. x 15 cm, 300 Å) and an HP 1090 liquid chromatographic system equipped with a diode-array detector and a workstation. The column was equilibrated with 0.1% trifluoroacetic acid (mobile phase A), and elution was effected with a linear gradient from 0%-55% mobile phase B (90% acetonitrile in 0.1% trifluoroacetic acid) over 70 min. The flow rate was 0.2 ml/min. and the column temperature was controlled at 25°C. One-third aliquots of the peptide peaks collected manually from the HPLC system were characterized by N-terminal sequence analysis by Edman degradation. The fraction eluted after 27.7 min. (T27.7) contained mixed amino acid sequences and was further rechromatographed after reduction as follows: A 70% aliquot of the peptide fraction was dried in vacuo and reconstituted in 100 μl of 0.2 M ammonium bicarbonate buffer (pH 7.8). DTT (final concentration 2 mM) was added to the solution, which was then incubated at 37°C for 30 min. The reduced peptide mixture was then separated by reverse-phase HPLC

using a Vydac column (2.1 mm i.d. x 15 cm). Elution conditions and flow rat'were identical to those described above. Amino acid sequence analysis of the peptide was performed with a Model 477 protein sequencer (Applied Biosystems, Inc., Foster City, CA) equipped with an on-line phenylthiohydantoin (PTH) amino acid analyzer and a Model 900 data analysis system (Hunkapiller et al. (1986) In Methods of Protein Microcharacterization, J.E. Shively, ed. (Clifton, New Jersey: Humana Press p. 223-247). The protein was loaded onto a trifluoroacetic acid-treated glass fiber disc precycled with polybrene and NaCl. The PTH-amino acid analysis was performed with a micro liquid chromatography system (Model 120) using dual syringe pumps and reverse-phase (C-18) narrow bore columns (Applied Biosystems, 2.1 mm x 250 mm).

RNA was isolated from Rat1-EJ cells by standard procedures (Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York (1982) and poly $(A)^+$ was selected using an mRNA Separator kit (Clontech Lab, Inc., Palo Alto, CA). cDNA was synthesized with the Superscript kit (from BRL Life Technologies, Inc., Bethesda, MD). Column-fractionated double-strand cDNA was ligated into an Sal1- and Not1-digested pJT-2 plasmid vector, a derivative of the pCD-X vector (Okayama and Berg, Mol. Cell Biol. 3: 280 (1983)) and transformed into DH10B E. coli cells by electroporation (Dower et al., Nucl. Acids Res. 16: 6127 (1988)). Approximately 5 x 10^5 primary transformants were screened with two oligonucleotide probes that were derived from the protein sequences of the N-terminus of NDF (residues 5-24) and the T40.4 tryptic peptide (residues 7-12). Their respective sequences were as follows (N indicates all 4 nt):

```
(1)  5'-ATA GGG AAG GGC GGG GGA AGG GTC NCC CTC NGC
       A     T
       AGG GCC GGG CTT GCC TCT GGA GCC TCT-3'
(2)  5'-TTT ACA CAT ATA TTC NCC-3'
       C   G         G   C
```

(1: SEQ ID No. 163; 2: SEQ ID No. 164)

The synthetic oligonucleotides were end-labeled with [γ-^{32}P]ATP with T4 polynucleotide kinase and used to screen replicate sets of nitrocellulose filters. The hybridization solution contained 6 x SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 2 x Denhardt's solution, 50 µg/ml salmon sperm DNA, and 20% formamide (for probe 1) or no formamide (for probe 2). The filters were washed at either 50°C with 0.5 x SSC, 0.2% SDS, 2 mM EDTA (for probe 1) or at 37°C with 2 x SSC, 0.2% SDS, 2 mM EDTA (for probe 2). Autoradiography of the filters gave ten clones that hybridized with both probes. These clones were purified by replating and probe hybridization as described above.

The cDNA clones were sequenced using an Applied Biosystems 373A automated DNA sequencer and Applied Biosystems Taq DyeDeoxy™ Terminator cycle sequencing kits following the manufacture's instructions. In some instances, sequences were obtained using [^{35}S]dATP (Amersham) and Sequenase™ kits from U.S. Biochemicals following the manufacturer's instructions. Both strands of the cDNA clone 44 were sequenced by using synthetic oligonucleotides as primers. The sequence of the most 5' 350 nt was determined in seven independent cDNA clones. The resultant clone demonstrated the pattern shown in Fig. 27 (NDF).

EXAMPLE 19

Purification and Assay of Other Proteins which bind $p185^{erbB2}$ Receptor

I. Purification of gp30 and p70

Lupu et al. (Science 249, 1552 (1990)) and Lippman and Lupu (patent application number PCT/US91/03443 (1990)), hereby incorporated by reference, have purified a protein from conditioned media of a human breast cancer cell line MDA-MB-231.

Lupu et al. (Proc. Natl. Acad. Sci. 89, 2287 (1992)) purified another protein which binds to the $p185^{erbB2}$ receptor. This particular protein, p75, was purified from conditioned medium used for the growth of SKBr-3 (a human breast cancer cell line) propagated in improved Eagle's medium (IMEM: GIBCO) supplemented with 10% fetal bovine serum (GIBCO).

II. Other $p185^{erbB2}$ ligands

Peles et al. (Cell 69, 205 (1992)) have also purified a 185^{erbB2} stimulating ligand from rat cells. Holmes et al. (Science 256, 1205 (1992)) have purified Heregulin α from human cells which binds and stimulates 185^{erbB2} (see Example 5). Tarakovsky et al. Oncogene 6:218 (1991) have demonstrated bending of a 25 kD polypeptide isolated from activated macrophages to the Neu receptor, a $p185^{erbB2}$ homology, herein incorporated by reference.

III. NDF Isolation

Yarden and Peles (Biochemistry 30, 3543 (1991)) have identified a 35 kilodalton glycoprotein which will stimulate the 185^{erbB2} receptor.

In other publications, Davis et al. (Biochem. Biophys. Res. Commun. 179, 1536 (1991), Proc. Natl. Acad. Sci. 88, 8582 (1991) and Greene et al., PCT patent application PCT/US91/02331 (1990)) describe the purification of a protein from conditioned medium of a human T-cell (ATL-2) cell line.

Huang et al. (1992, J. Biol. Chem. 257:11508-11512), hereby incorporated by reference, have isolated an additional neu/erb B2 ligand growth factor from bovine kidney. The 25 kD polypeptide factor was isolated by a procedure of column fractionation, followed by sequential column chromatography on DEAE/cellulose (DE52), Sulfadex (sulfated Sephadex G-50), heparin-Sepharose 4B, and Superdex 75 (fast protein liquid chromatography). The factor, NEL-GF, stimulates tyrosine-specific autophosphorylation of the neu/erb B2 gene product.

IV. Purification of acetylcholine receptor inducing activity (ARIA)

ARIA, a 42 kD protein which stimulates acetylcholine receptor synthesis, has been isolated in the laboratory of Gerald Fischbach (Falls et al., (1993) Cell 72:801-815). ARIA induces tyrosine phosphorylation of a 185 Kda muscle transmembrane protein which resembles $p185^{erbB2}$, and stimulates acetylcholine receptor synthesis in cultured embryonic myotubes. ARIA is most likely a member of the GGF/erbB2 ligand group of proteins, and this is potentially useful in the glial cell mitogenesis stimulation and other applications of, e.g., GGF2 described herein.

EXAMPLE 19

Protein tyrosine phosphorylation mediated by GGF

Rat Schwann cells, following treatment with sufficient levels of Glial Growth Factor to induce proliferation, show stimulation of protein tyrosine phosphorylation. Varying amounts of partially purified GGF were applied to a primary culture of rat Schwann cells according to the procedure outlined in Example 9. Schwann cells were grown in DMEM/10% fetal calf serum/5 μM forskolin/0.5μg per mL GGF-CM (0.5mL per well) in poly D-lysine coated 24 well plates. When confluent, the cells were fed with DMEM/10% fetal calf serum at 0.5mL per well and left in the incubator overnight to quiesce. The following day, the cells were fed with 0.2mL of DMEM/10% fetal calf serum and left in the incubator for 1 hour. Test samples were then added directly to the medium at different concentrations and for different lengths of time as required. The cells were then lysed in boiling lysis buffer (sodium phosphate, 5mM, pH 6.8; SDS, 2%, β-mercapteothanol, 5%; dithiothreitol, 0.1M; glycerol, 10%; Bromophenol Blue, 0.4%; sodium vanadate, 10mM), incubated in a boiling water bath for 10 minutes and then either analyzed directly or frozen at -70°C. Samples were analyzed by running on 7.5% SDS-PAGE gels and then electroblotting onto nitrocellulose using standard procedures as described by Towbin et al. (1979) Proc. Natl. Acad. Sci. USA 76:4350-4354. The blotted nitrocellulose was probed with antiphosphotyrosine antibodies using standard methods as described in Kamps and Selton (1988) Oncogene 2:305-315. The probed blots were exposed to autoradiography film overnight and developed using a standard laboratory processor. Densitometric measurements were carried out using an Ultrascan XL enhanced laser densitometer (LKB).

Molecular w ight assignments w re mad relative t prestained high molecular weight standards (Sigma). The dose responses of protein phosphorylation and Schwann cell proliferation are very similar (Fig. 33). The molecular weight of the phosphorylated band is very close to the molecular weight of p185^{erbB2}. Similar results were obtained when Schwann cells were treated with conditioned media prepared from COS cells translates with the GGF2HBS5 clone. These results correlate well with the expected interaction of the GGFs with and activation of 185^{erbB2}.

This experiment has been repeated with recombinant GGF2. Conditioned medium derived from a CHO cell line stably transformed with the GGF2 clone (GGF2HBS5) stimulates protein tyrosine phosphorylation using the assay described above. Mock transfected CHO cells fail to stimulate this activity.

EXAMPLE 20

N-glycosylation of GGF

The protein sequence predicted from the cDNA sequence of GGF-II candidate clones GGF2BPP1,2 and 3 contains a number of consensus N-glycosylation motifs. A gap in the GGFII02 peptide sequence coincides with the asparagine residue in one of these motifs, indicating that carbohydrate is probably bound at this site.

N-glycosylation of the GGFs was studied by observing mobility changes on SDS-PAGE after incubation with N-glycanase, an enzyme that cleaves the covalent linkages between carbohydrate and aspargine residues in proteins.

N-Glycanase treatment of GGF-II yielded a major band of MW 40-42 kDa and a minor band at 45-48 kDa.

Activity singl active d glycosylated species at ca 45-50 kDa.

Activity elution experiments with GGF-I also demonstrate an increase in electrophoretic mobility when treated with N-Glycanase, giving an active species of MW 26-28 kDa. Silver staining confirmed that there is a mobility shift, although no N-deglycosylated band could be assigned because of background staining in the sample used.

(1) GENERAL INFORMATION:

(i) APPLICANTS: Robert Sklar, Mark Marchionni, David I. Gwynne

(ii) TITLE OF INVENTION: METHODS FOR ALTERING MUSCLE CONDITION

(iii) NUMBER OF SEQUENCES: 185

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 94/05083 A
(B) FILING DATE: 06-MAY-94
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/209,204
(B) FILING DATE: 08-MAR-94

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/059,022
(B) FILING DATE: 06-May-93

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 04585/028WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(B) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Phe Lys Gly Asp Ala His Thr Glu
 1               5
```

(2) INFORMATI N FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amin acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine; Xaa in position 12 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Xaa Ala Ser Leu Ala Asp Glu Tyr Glu Tyr Met Xaa Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine; Xaa in position 10 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Xaa Thr Glu Thr Ser Ser Ser Gly Leu Xaa Leu Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Xaa Lys Leu Gly Glu Met Trp Ala Glu
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Xaa Leu Gly Glu Lys Arg Ala
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Xaa Ile Lys Ser Glu His Ala Gly Leu Ser Ile Gly Asp Thr Ala Lys
 1               5                  10                  15
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Xaa Ala Ser Leu Ala Asp Glu Tyr Glu Tyr Met Arg Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTI N: SEQ ID NO: 8:

Xaa Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine and Xaa in position 12 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Xaa Met Ser Glu Tyr Ala Phe Phe Val Gln Thr Xaa Arg
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Xaa Ser Glu His Pro Gly Leu Ser Ile Gly Asp Thr Ala Lys
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine; Xaa in position 8 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Xaa Ala Gly Tyr Phe Ala Glu Xaa Ala Arg
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine; Xaa in position 7 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Xaa Lys Leu Glu Phe Leu Xaa Ala Lys
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Xaa Thr Thr Glu Met Ala Ser Glu Gln Gly Ala
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Xaa Ala Lys Glu Ala Leu Ala Ala Leu Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATI N: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Xaa Phe Val Leu Gln Ala Lys Lys
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Xaa Leu Gly Glu Met Trp
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Glu Tyr Lys Cys Leu Lys Phe Lys Trp Phe Lys Lys Ala Thr Val Met
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 8 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Ala Lys Tyr Phe Ser Lys Xaa Asp Ala
1 5 10

(2) INFORMATI N FOR SEQUENCE IDENTIFICATION NUMBER: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 2 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Glu Xaa Lys Phe Tyr Val Pro
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Glu Leu Ser Phe Ala Ser Val Arg Leu Pro Gly Cys Pro Pro Gly Val
1 5 10 15

Asp Pro Met Val Ser Phe Pro Val Ala Leu
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2003
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N in positions 31 and 32 could be either A or G.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGAATTCCTT TTTTTTTTTT TTTTTTTCTT NNTTTTTTTT TGCCCTTATA CCTCTTCGCC 60

TTTCTGTGGT TCCATCCACT TCTTCCCCCT CCTCCTCCCA TAAACAACTC TCCTACCCCT 120

GCACCCCCAA TAAATAAATA AAAGGAGGAG GGCAAGGGGG GAGGAGGAGG AGTGGTGCTG 180

CGAGGGGAAG GAAAAGGGAG GCAGCGCGAG AAGAGCCGGG CAGAGTCCGA ACCGACAGCC 240

AGAAGCCCGC ACGCACCTCG CACC ATG AGA TGG CGA CGC GCC CCG CGC CGC 291
Met Arg Trp Arg Arg Ala Pro Arg Arg
1 5

TCC GGG CGT CCC GGC CCC CGG GCC CAG CGC CCC GGC TCC GCC GCC CGC 339
Ser Gly Arg Pro Gly Pro Arg Ala Gln Arg Pro Gly Ser Ala Ala Arg
10 15 20 25

```
TCG TCG CCG CCG CTG CCG CTG CTG CCA CTA CTG CTG CTG CTG GGC ACC      387
Ser Ser Pro Pro Leu Pro Leu Leu Pro Leu Leu Leu Leu Leu Gly Thr
                30                  35                  40

GCG GCC CTG GCG CCG GGG GCG GCG GCC GGC AAC GAG GCG GCT CCC GCG      435
Ala Ala Leu Ala Pro Gly Ala Ala Ala Gly Asn Glu Ala Ala Pro Ala
            45                  50                  55

GGG GCC TCG GTG TGC TAC TCG TCC CCG CCC AGC GTG GGA TCG GTG CAG      483
Gly Ala Ser Val Cys Tyr Ser Ser Pro Pro Ser Val Gly Ser Val Gln
        60                  65                  70

GAG CTA GCT CAG CGC GCC GCG GTG GTG ATC GAG GGA AAG GTG CAC CCG      531
Glu Leu Ala Gln Arg Ala Ala Val Val Ile Glu Gly Lys Val His Pro
    75                  80                  85

CAG CGG CGG CAG CAG GGG GCA CTC GAC AGG AAG GCG GCG GCG GCG GCG      579
Gln Arg Arg Gln Gln Gly Ala Leu Asp Arg Lys Ala Ala Ala Ala Ala
90                  95                  100                 105

GGC GAG GCA GGG GCG TGG GGC GGC GAT CGC GAG CCG CCA GCC GCG GGC      627
Gly Glu Ala Gly Ala Trp Gly Gly Asp Arg Glu Pro Pro Ala Ala Gly
                110                 115                 120

CCA CGG GCG CTG GGG CCG CCC GCC GAG GAG CCG CTG CTC GCC GCC AAC      675
Pro Arg Ala Leu Gly Pro Pro Ala Glu Glu Pro Leu Leu Ala Ala Asn
            125                 130                 135

GGG ACC GTG CCC TCT TGG CCC ACC GCC CCG GTG CCC AGC GCC GGC GAG      723
Gly Thr Val Pro Ser Trp Pro Thr Ala Pro Val Pro Ser Ala Gly Glu
        140                 145                 150

CCC GGG GAG GAG GCG CCC TAT CTG GTG AAG GTG CAC CAG GTG TGG GCG      771
Pro Gly Glu Glu Ala Pro Tyr Leu Val Lys Val His Gln Val Trp Ala
    155                 160                 165

GTG AAA GCC GGG GGC TTG AAG AAG GAC TCG CTG CTC ACC GTG CGC CTG      819
Val Lys Ala Gly Gly Leu Lys Lys Asp Ser Leu Leu Thr Val Arg Leu
170                 175                 180                 185

GGG ACC TGG GGC CAC CCC GCC TTC CCC TCC TGC GGG AGG CTC AAG GAG      867
Gly Thr Trp Gly His Pro Ala Phe Pro Ser Cys Gly Arg Leu Lys Glu
                190                 195                 200

GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC GAC GCC AAC AGC ACC AGC      915
Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Asp Ala Asn Ser Thr Ser
            205                 210                 215

CGC GCG CCG GCC GCC TTC CGA GCC TCT TTC CCC CCT CTG GAG ACG GGC      963
Arg Ala Pro Ala Ala Phe Arg Ala Ser Phe Pro Pro Leu Glu Thr Gly
        220                 225                 230

CGG AAC CTC AAG AAG GAG GTC AGC CGG GTG CTG TGC AAG CGG TGC GCC     1011
Arg Asn Leu Lys Lys Glu Val Ser Arg Val Leu Cys Lys Arg Cys Ala
    235                 240                 245

TTG CCT CCC CAA TTG AAA GAG ATG AAA AGC CAG GAA TCG GCT GCA GGT     1059
Leu Pro Pro Gln Leu Lys Glu Met Lys Ser Gln Glu Ser Ala Ala Gly
250                 255                 260                 265

TCC AAA CTA GTC CTT CGG TGT GAA ACC AGT TCT GAA TAC TCC TCT CTC     1107
Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
                270                 175                 180

AGA TTC AAG TGG TTC AAG AAT GGG AAT GAA TTG AAT CGA AAA AAC AAA     1155
Arg Phe Lys Trp Phe Lys Asn Gly Asn Glu Leu Asn Arg Lys Asn Lys
            185                 190                 195
```

CCA CAA AAT ATC AAG ATA CAA AAA AAG CCA GGG AAG TCA GAA CTT CGC 1203
Pro Gln Asn Ile Lys Ile Gln Lys Lys Pr Gly Lys Ser Glu Leu Arg
200 205 210

ATT AAC AAA GCA TCA CTG GCT GAT TCT GGA GAG TAT ATG TGC AAA GTG 1251
Ile Asn Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val
215 220 225

ATC AGC AAA TTA GGA AAT GAC AGT GCC TCT GCC AAT ATC ACC ATC GTG 1299
Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val
230 235 240 245

GAA TCA AAC GCT ACA TCT ACA TCC ACC ACT GGG ACA AGC CAT CTT GTA 1347
Glu Ser Asn Ala Thr Ser Thr Ser Thr Thr Gly Thr Ser His Leu Val
250 255 260

AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC 1395
Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
265 270 275

TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG TGC AAG TGC 1443
Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys
280 285 290

CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC 1491
Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser
295 300 305

TTC TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA 1530
Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu
400 405 410

TAGGAGCATG CTCAGTTGGT GCTGCTTTCT TGTTGCTGCA TCTCCCCTCA GATTCCACCT 1590

AGAGCTAGAT GTGTCTTACC AGATCTAATA TTGACTGCCT CTGCCTGTCG CATGAGAACA 1650

TTAACAAAAG CAATTGTATT ACTTCCTCTG TTCGCGACTA GTTGGCTCTG AGATACTAAT 1710

AGGTGTGTGA GGCTCCGGAT GTTTCTGGAA TTGATATTGA ATGATGTGAT ACAAATTGAT 1770

AGTCAATATC AAGCAGTGAA ATATGATAAT AAAGGCATTT CAAAGTCTCA CTTTTATTGA 1830

TAAAATAAAA ATCATTCTAC TGAACAGTCC ATCTTCTTTA TACAATGACC ACATCCTGAA 1890

AAGGGTGTTG CTAAGCTGTA ACCGATATGC ACTTGAAATG ATGGTAAGTT AATTTTGATT 1950

CAGAATGTGT TATTTGTCAC AAATAAACAT AATAAAAGGA AAAAAAAAAA AAA 2003

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 11 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Ala Ser Leu Ala Asp Glu Tyr Glu Tyr Met Xaa Lys
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 9 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
Thr Glu Thr Ser Ser Ser Gly Leu Xaa Leu Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
Ala Ser Leu Ala Asp Glu Tyr Glu Tyr Met Arg Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 7 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
Ala Gly Tyr Phe Ala Glu Xaa Ala Arg
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Thr Thr Glu Met Ala Ser Glu Gln Gly Ala
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
Ala Lys Glu Ala Leu Ala Ala Leu Lys
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

```
Phe Val Leu Gln Ala Lys Lys
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```
Glu Thr Gln Pro Asp Pro Gly Gln Ile Leu Lys Lys Val Pro Met Val
 1               5                  10                  15

Ile Gly Ala Tyr Thr
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in positions 1, 3, 17 and 19 is unknown.

(xi) SEQUENCE DESCRIPTI N: SEQ ID N : 30:

```
Xaa Glu Xaa Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys Lys Glu
 1               5                  10                  15
```

Xaa ly Xaa Gly Lys
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 6 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Lys Leu Glu Phe Leu Xaa Ala Lys
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Xaa Val His Gln Val Trp Ala Ala Lys
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATI N: Xaa in position 1 is Lysine or Arginine, Xaa in position 11 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

```
Xaa Tyr Ile Phe Phe Met Glu Pro Glu Ala Xaa Ser Ser Gly
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine, Xaa in position 13 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```
Xaa Leu Gly Ala Trp Gly Pro Pro Ala Phe Pro Val Xaa Tyr
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```
Xaa Trp Phe Val Val Ile Glu Gly Lys
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in positi n 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Xaa Ala Ser Pr Val Ser Val Gly Ser Val Gln Glu L u Val Gln Arg
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Xaa Val Cys Leu Leu Thr Val Ala Ala Leu Pro Pro Thr
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is Lysine or Arginine; Xaa in position 6 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Xaa Asp Leu Leu Leu Xaa Val
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Cys Thr Cys Gly Cys Cys Lys Cys Cys Arg Thr Thr Cys Ala Cys Arg
1 5 10 15

Cys Ala Gly Ala Ala Gly Gly Thr Cys Thr Thr Cys Thr Cys Cys Thr
20 25 30

Thr Cys Thr Cys Ala Gly Cys
35

(2) INFORMATION FOR SEQUENCE IDENTIFICATI N NUMBER: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

```
Cys Cys Thr Cys Gly Cys Thr Cys Cys Thr Thr Cys Thr Thr Cys Thr
 1               5                  10                  15

Thr Gly Cys Cys Cys Thr Thr Cys
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```
Val His Gln Val Trp Ala Ala Lys
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 10 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```
Tyr Ile Phe Phe Met Glu Pro Glu Ala Xaa Ser Ser Gly
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 12 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Leu Gly Ala Trp Gly Pro Pro Ala Phe Pro Val Xaa Tyr
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

Trp Phe Val Val Ile Glu Gly Lys
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Ala Ser Pro Val Ser Val Gly Ser Val Gln Glu Leu Val Gln Arg
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Val Cys Leu Leu Thr Val Ala Ala Leu Pro Pro Thr
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Lys Val His Gln Val Trp Ala Ala Lys
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 12 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Xaa Lys
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 5 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Asp Leu Leu Leu Xaa Val
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

TTYAARGGNG AYGCNCAYAC 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

CATRTAYTCR TAYTCRTCNG C 21

(2) INFORMATI N FOR SEQUENCE IDENTIFICATION NUMBER: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

TGYTCNGANG CCATYTCNGT 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

TGYTCRCTNG CCATYTCNGT 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

CCDATNACCA TNGGNACYTT 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GCNGCCCANA CYTGRTGNAC 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GCYTCNGGYT CCATRAARAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

CCYTCDATNA CNACRAACCA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

TCNGCRAART ANCCNGC 17

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

GCNGCNAGNG CYTCYTTNGC 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GCNGCYAANG CYTCYTTNGC 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

TTYTTNGCYT GNAGNACRAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

TTYTTNGCYT GYAANACRAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

TGNACNAGYT CYTGNAC 17

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

TGNACYAAYT CYTGNAC 17

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

CATRTAYTCN CCNGARTCNG C 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID N : 67:

CATRTAYTCN CCRCTRTCNG C 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

NGARTCNGCY AANGANGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

NGARTCNGCN AGNGANGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

RCTRTCNGCY AANGANGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

RCTRTCNGCN AGNGANGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

NGARTCNGCY AARCTNGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

NGARTCNGCN AGRCTNGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

RCTRTCNGCY AARCTNGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

RCTRCTNGCN AGRCTNGCYT T 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

ACNACNGARA TGGCTCNNGA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

ACNACNGARA TGGCAGYNGA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

CAYCARGTNT GGGCNGCNAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

TTYGTNGTNA THGARGGNAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

AARGGNGAYG CNCAYACNGA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

GARGCNYTNG CNGCNYTNAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

GTNGGNTCNG TNCARGARYT 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

GTNGGNAGYG TNCARGARYT 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

NACYTTYTTN ARDATYTGNC C 21

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 417
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in positions 14, 23, 90, 100, 126, and 135 is a stop codon.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

```
TCTAA AAC TAC AGA GAC TGT ATT TTC ATG ATC ATC ATA GTT CTG TGA AAT ATA    53
      Asn Tyr Arg Asp Cys Ile Phe Met Ile Ile Ile Val Leu Xaa Asn Ile
        1               5                  10                  15

CTT AAA CCG CTT TGG TCC TGA TCT TGT AGG AAG TCA GAA CTT CGC ATT         101
Leu Lys Pro Leu Trp Ser Xaa Ser Cys Arg Lys Ser Glu Leu Arg Ile
            20                  25                  30

AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC         149
Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Ser Met Cys Lys Val Ile
        35                  40                  45
```

```
AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG        197
Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Arg Ile Val Glu
    50                  55                  60

TCA AAC GGT AAG AGA TGC CTA CTG CGT GCT ATT TCT CAG TCT CTA AGA        245
Ser Asn Gly Lys Arg Cys Leu Leu Arg Ala Ile Ser Gln Ser Leu Arg
65                  70                  75                  80

GGA GTG ATC AAG GTA TGT GGT CAC ACT TGA ATC ACG CAG GTG TGT GAA        293
Gly Val Ile Lys Val Cys Gly His Thr Xaa Ile Thr Gln Val Cys Glu
                85                  90                  95

ATC TCA TTG TGA ACA AAT AAA AAT CAT GAA AGG AAA ACT CTA TGT TTG        341
Ile Ser Cys Xaa Thr Asn Lys Asn His Glu Arg Lys Thr Leu Cys Leu
            100                 105                 110

AAA TAT CTT ATG GGT CCT CCT GTA AAG CTC TTC ACT CCA TAA GGT GAA        389
Lys Tyr Leu Met Gly Pro Pro Val Lys Leu Phe Thr Pro Xaa Gly Glu
        115                 120                 125

ATA GAC CTG AAA TAT ATA TAG ATT ATT T                                  417
Ile Asp Leu Lys Tyr Ile Xaa Ile Ile
    130                 135
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 19, 25, and 31 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

CCGAATTCTG CAGGARACNC ARCCNGAYCC NGG 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 87:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 14, 20, 23, 29, and 35 is Inosine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

AAGGATCCTG CAGNGTRTAN GCNCCDATNA CCATNGG 37

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 16, 21, and 24 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

CCGAATTCTG CAGGCNGAYT CNGGNGARTA YATG 34

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 16 and 25 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

CCGAATTCTG CAGGCNGAYA GYGGNGARTA YAT 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 14, 15, 16, 26, and 29 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

AAGGATCCTG CAGNNNCATR TAYTCNCCNG ARTC 34

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 14, 15, 16, and 26 is Inosine. Y can be cytidine r thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

AAGGATCCTG CAGNNNCATR TAYTCNCCRC TRTC 34

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 21, 28, and 31 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

CCGAATTCTG CAGCAYCARG TNTGGGCNGC NAA 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 93:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 31 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

CCGAATTCTG CAGATHTTYT TYATGGARCC NGARG 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 94:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 18, 21, 24, 27, and 33 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

CCGAATTCTG CAGGGGGNCC NCCNGCNTTY CCNGT 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 95:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 21 and 24 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

CCGAATTCTG CAGTGGTTYG TNGTNATHGA RGG 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 96:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 17, 20, and 26 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96

AAGGATCCTG CAGYTTNGCN GCCCANACYT GRTG 34

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 19 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

AAGGATCCTG CAGGCYTCNG GYTCCATRAA RAA 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 16, 22, 25, 28, and 31 is Inosine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

AAGGATCCTG CAGACNGGRA ANGCNGGNGG NCC 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at positions 17, 26, and 29 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

AAGGATCCTG CAGYTTNCCY TCDATNACNA CRAAC 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 18 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

CATRTAYTCR TAYTCTCNGC AAGGATCCTG CAG 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 101:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 19, 25, and 31 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

CCGAATTCTG CAGAARGGNG AYGCNCAYAC NGA 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 102:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 3 and 18 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

GCNGCYAANG CYTCYTTNGC AAGGATCCTG CAG 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 103:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 3, 6, 9, and 18 is Inosine. Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

GCNGCNAGNG CYTCYTTNGC AAGGATCCTG CAG 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 104:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N at position 3, 12, and 15 is Inosine.Y can be cytidine or thymidine.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

TCNGCRAART ANCCNGCAAG GATCCTGCAG 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

CATCGATCTG CAGGCTGATT CTGGAGAATA TATGTGCA 38

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 106:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

AAGGATCCTG CAGCCACATC TCGAGTCGAC ATCGATT 37

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 107:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

CCGAATTCTG CAGTGATCAG CAAACTAGGA AATGACA 37

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 108:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

CATCGATCTG CAGCCTAGTT TGCTGATCAC TTTGCAC 37

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 109:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

AAGGATCCTG CAGTATATTC TCCAGAATCA GCCAGTG 37

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 110:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

AAGGATCCTG CAGGCACGCA GTAGGCATCT CTTA 34

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 111:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

CCGAATTCTG CAGCAGAACT TCGCATTAGC AAAGC 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 112:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

CATCCCGGGA TGAAGAGTCA GGAGTCTGTG GCA 33

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 113:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

ATACCCGGGC TGCAGACAAT GAGATTTCAC ACACCTGCG 39

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 114:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

AAGGATCCTG CAGTTTGGAA CCTGCCACAG ACTCCT 36

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 115:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

ATACCCGGGC TGCAGATGAG ATTTCACACA CCTGCGTGA 39

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 116:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

```
His Gln Val Trp Ala Ala Lys Ala Ala Gly Leu Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 117:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

```
Gly Gly Leu Lys Lys Asp Ser Leu Leu Thr Val Arg Leu Gly Ala Asn
 1               5                  10                  15
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 118:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 12 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

```
Leu Gly Ala Trp Gly Pro Pro Ala Phe Pro Val Xaa Tyr
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 119:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

```
Leu Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser
 1               5                  10                  15

Cys Gly Arg Leu Lys Glu Asp
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 120:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 10 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

```
Tyr Ile Phe Phe Met Glu Pro Glu Ala Xaa Ser Ser Gly
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 121:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

```
Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Glu Ala Asn Ser
 1               5                  10                  15

Ser Gly Gly Pro Gly Arg Leu
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 122:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

```
Val Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 123:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

```
Glu Tyr Lys Cys Leu Lys Phe Lys Trp Phe Lys Lys Ala Thr Val Met
 1               5                  10                  15
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 124:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

```
Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu Lys Phe Lys Trp Phe Lys
 1               5                  10                  15

Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys
            20                  25
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 125:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 12 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

```
Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Xaa Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 126:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

```
Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met
 1               5                  10                  15

Cys Lys Val Ile Ser Lys Leu
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 127:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

```
Ala Ser Leu Ala Asp Glu Tyr Glu Tyr Met Arg Lys
 1               5                  10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 128:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

```
Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys
 1               5                  10                  15

Lys Val Ile Ser Lys Leu
             20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 129:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 744
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:

```
CCTGCAG CAT CAA GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG CTG   55
        His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser Leu
         1               5                  10                  15

CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC TGC          103
Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser Cys
            20                  25                  30

GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC GAG          151
Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Glu
        35                  40                  45

GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC CCC          199
Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro Pro
    50                  55                  60

TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT GTG          247
Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala Val
65                  70                  75                  80

CAA CGG TGC GCC TTG CCT CCC CGC TT  AAA GAG ATG AAG AGT CAG GAG          295
Gln Arg Cys Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
                85                  90                  95
```

```
TCT GTG GCA GGT TCC AAA CTA GTG CTT CGG TGC GAG ACC AGT TCT GAA          343
Ser Val Ala  ly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
        100                 105                 110

TAC TCC TCT CTC AAG TTC AAG TGG TTC AAG AAT GGG AGT GAA TTA AGC          391
Tyr Ser Ser Leu Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser
    115                 120                 125

CGA AAG AAC AAA CCA GAA AAC ATC AAG ATA CAG AAA AGG CCG GGG AAG          439
Arg Lys Asn Lys Pro Glu Asn Ile Lys Ile Gln Lys Arg Pro Gly Lys
    130                 135                 140

TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA TAT          487
Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr
145                 150                 155                 160

ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC AAC          535
Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn
                165                 170                 175

ATC ACC ATT GTG GAG TCA AAC GGT AAG AGA TGC CTA CTG CGT GCT ATT          583
Ile Thr Ile Val Glu Ser Asn Gly Lys Arg Cys Leu Leu Arg Ala Ile
            180                 185                 190

TCT CAG TCT CTA AGA GGA GTG ATC AAG GTA TGT GGT CAC ACT                  625
Ser Gln Ser Leu Arg Gly Val Ile Lys Val Cys Gly His Thr
        195                 200                 205

TGAATCACGC AGGTGTGTGA AATCTCATTG TGAACAAATA AAAATCATGA AAGGAAAAAA        685

AAAAAAAAAA AATCGATGTC GACTCGAGAT GTGGCTGCAG GTCGACTCTA GAGGATCCC         744
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 130:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1193
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

```
CCTGCAG CAT CAA GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG CTG  55
        His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser Leu
        1               5                   10                  15

CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC TGC          103
Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser Cys
            20                  25                  30

GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC GAG          151
Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Glu
        35                  40                  45

GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC CCC          199
Ala Lys Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro Pro
    50                  55                  60

TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT GTG          247
Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala Val
65                  70                  75                  80
```

```
CAA CGG TGC GCC TTG CCT CCC CGC TTG AAA GAG ATG AAG AGT CAG GAG      295
Gln Arg Cys Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
                 85                  90                  95

TCT GTG GCA GGT TCC AAA CTA GTG CTT CGG TGC GAG ACC AGT TCT GAA      343
Ser Val Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
            100                 105                 110

TAC TCC TCT CTC AAG TTC AAG TGG TTC AAG AAT GGG AGT GAA TTA AGC      391
Tyr Ser Ser Leu Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser
        115                 120                 125

CGA AAG AAC AAA CCA GAA AAC ATC AAG ATA CAG AAA AGG CCG GGG AAG      439
Arg Lys Asn Lys Gly Gly Asn Ile Lys Ile Gln Lys Arg Pro Gly Lys
    130                 135                 140

TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA TAT      487
Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr
145                 150                 155                 160

ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC AAC      535
Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn
                165                 170                 175

ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG ACA      583
Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly Thr
            180                 185                 190

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT      631
Ser His Leu Val Lys Ser Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
        195                 200                 205

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC      679
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
    210                 215                 220

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT      727
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
225                 230                 235                 240

GTG CCC ATG AAA GTC CAA ACC CAA GAA AGT GCC CAA ATG AGT TTA CTG      775
Val Pro Met Lys Val Gln Thr Gln Glu Ser Ala Gln Met Ser Leu Leu
                245                 250                 255

GTG ATC GCT GCC AAA ACT ACG TAATGGCCAG CTTCTACAGT ACGTCCACTC         826
Val Ile Ala Ala Lys Thr Thr
            260

CCTTTCTGTC TCTGCCTGAA TAGCGCATCT CAGTCGGTGC CGCTTTCTTG TTGCCGCATC    886

TCCCCTCAGA TTCCTCCTAG AGCTAGATGC GTTTTACCAG GTCTAACATT GACTGCCTCT    946

GCCTGTCGCA TGAGAACATT AACACAAGCG ATTGTATGAC TTCCTCTGTC CGTGACTAGT   1006

GGGCTCTGAG CTACTCGTAG GTGCGTAAGG CTCCAGTGTT TCTGAAATTG ATCTTGAATT   1066

ACTGTGATAC GACATGATAG TCCCTCTCAC CCAGTGCAAT GACAATAAAG GCCTTGAAAA   1126

GTCAAAAAAA AAAAAAAAAA AAAAAATCGA TGTCGACTCG AGATGTGGCT GCAGGTCGAC   1186

TCTAGAG                                                              1193
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 131:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1108
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

```
CCTGCAG CAT CAA GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG CTG   55
        His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser Leu
        1               5                   10                  15

CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC TGC          103
Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser Cys
        20                  25                  30

GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC GAG          151
Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Glu
        35                  40                  45

GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC CCC          199
Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro Pro
    50                  55                  60

TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT GTG          247
Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala Val
65                  70                  75                  80

CAA CGG TGC GCC TTG CCT CCC CGC TTG AAA GAG ATG AAG AGT CAG GAG          295
Gln Arg Cys Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
            85                  90                  95

TCT GTG GCA GGT TCC AAA CTA GTG CTT CGG TGC GAG ACC AGT TCT GAA          343
Ser Val Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
        100                 105                 110

TAC TCC TCT CTC AAG TTC AAG TGG TTC AAG AAT GGG AGT GAA TTA AGC          391
Tyr Ser Ser Leu Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser
        115                 120                 125

CGA AAG AAC AAA CCA GAA AAC ATC AAG ATA CAG AAA AGG CCG GGG AAG          439
Arg Lys Asn Lys Pro Glu Asn Ile Lys Ile Gln Lys Arg Pro Pro Lys
    130                 135                 140

TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA TAT          487
Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr
145                 150                 155                 160

ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC AAC          535
Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn
            165                 170                 175

ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG ACA          583
Ile Arg Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly Thr
        180                 185                 190

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT          631
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
    195                 200                 205

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC          679
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
    210                 215                 220

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC          727
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr
225                 230                 235                 240
```

GTA ATG GCC AGC TTC TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT 775
Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pr
245 250 255

GAA TAGCGCATCT CAGTCGGTGC CGCTTTCTTG TTGCCGCATC TCCCCTCAGA TTCCGCCTAG 838
Glu

AGCTAGATGC GTTTTACCAG GTCTAACATT GACTGCCTCT GCCTGTCGCA TGAGAACATT 898

AACACAAGCG ATTGTATGAC TTCCTCTGTC CGTGACTAGT GGGCTCTGAG CTACTCGTAG 958

GTGCGTAAGG CTCCAGTGTT TCTGAAATTG ATCTTGAATT ACTGTGATAC GACATGATAG 1018

TCCCTCTCAC CCAGTGCAAT GACAATAAAG GCCTTGAAAA GTCAAAAAAA AAAAAAAAAA 1078

AAAAATCGAT GTCGACTCGA GATGTGGCTG 1108

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 132:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 559
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N in position 214 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:

AGTTTCCCCC CCCAACTTGT CGGAACTCTG GGCTCGCCCG CAGGGCAGGA GCGGAGCGGC 60

GGCGGCTGCC CAGGCGATGC GAGCGCGGGC CGGACGGTAA TCGCCTCTCC CTCCTCGGGC 120

TGCGAGCGCG CCGGACCGAG GCAGCGACAG GAGCGGACCG CGGCGGGAAC CGAGGACTCC 180

CCAGCGGCGC GCCAGCAGGA GCCACCCCGC GAGNCGTGCG ACCGGGACGG AGCGCCCGCC 240

AGTCCCAGGT GGCCCGGACC GCACGTTGCG TCCCCGCGCT CCCCGCCGGC GACAGGAGAC 300

GCTCCCCCCC ACGCCGCGCG CGCCTCGGCC CGGTCGCTGG CCCGCCTCCA CTCCGGGGAC 360

AAACTTTTCC CGAAGCCGAT CCCAGCCCTC GGACCCAAAC TTGTCGCGCG TCGCCTTCGC 420

CGGGAGCCGT CCGCGCAGAG CGTGCACTTC TCGGGCGAG ATG TCG GAG CGC AGA 474
Met Ser Glu Arg Arg
1 5

GAA GGC AAA GGC AAG GGG AAG GGC GGC AAG AAG GAC CGA GGC TCC GGG 522
Glu Gly Lys Gly Lys Gly Lys Gly Gly Lys Lys Asp Arg Gly Ser Gly
10 15 20

AAG AAG CCC GTG CCC GCG GCT GGC GGC CCG AGC CCA G 559
Lys Lys Pro Val Pro Ala Ala Gly Gly Pro Ser Pro Ala
25 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 133:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 252
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N in position 8 could be either A or G.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:

```
CC CAT CAN GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG        47
   His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser
    1               5                  10                  15

CTG CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC       95
Leu Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser
                20                  25                  30

TGC GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC      143
Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro
            35                  40                  45

GAG GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC      191
Glu Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro
        50                  55                  60

CCC TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT      239
Pro Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala
    65                  70                  75

GTG CAA CGG TGC G                                                    252
Val Gln Arg Cys
 80
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 134:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 178
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

```
CCT TGC CTC CCC GCT TGA AAG AGA TGA AGA GTC AGG AGT CTG TGG CAG       48
Leu Pro Pro Arg Leu Lys Glu His Lys Ser Gln Glu Ser Val Ala Gly
 1               5                  10                  15

GTT CCA AAC TAG TGC TTC GGT GCG AGA CCA GTT CTG AAT ACT CCT CTC       96
Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu
            20                  25                  30

TCA AGT TCA AGT GGT TCA AGA ATG GGA GTG AAT TAA GCC GAA AGA ACA      144
Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys
        35                  40                  45

AAC CAC AAA ACA TCA AGA TAC AGA AAA GGC CGG G                        178
Pro Gly Asn Ile Lys Ile Gln Lys Arg Pro Gly
    50                  55
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATI N NUMBER: 135:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

```
G AAG TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA        46
  Lys Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly
   1               5                  10                  15

GAA TAT ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT      94
Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser
                20                  25                  30

GCC AAC ATC ACC ATT GTG GAG TCA AAC G                                122
Ala Asn Ile Thr Ile Val Glu Ser Asn Ala
            35
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 136:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 417
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

```
TCTAAAACTA CAGAGACTGT ATTTTCATGA TCATCATAGT TCTGTGAAAT ATACTTAAAC     60

CGCTTTGGTC CTGATCTTGT AGG AAG TCA GAA CTT CGC ATT AGC AAA GCG        110
                          Lys Ser Glu Leu Arg Ile Ser Lys Ala
                           1               5

TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC AGC AAA CTA      158
Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu
 10                  15                  20                  25

GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG TCA AAC GGT      206
Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Gly
                30                  35                  40

AAG AGA TGC CTA CTG CGT GCT ATT TCT CAG TCT CTA AGA GGA GTG ATC      254
Lys Arg Cys Leu Leu Arg Ala Ile Ser Gln Ser Leu Arg Gly Val Ile
            45                  50                  55

AAG GTA TGT GGT CAC ACT TGAATCACGC AGGTGTGTGA AATCTCATTC             302
Lys Val Cys Gly His Thr
        60

TGAACAAATA AAAATCATGA AAGGAAAACT CTATCTTTGA AATATCTTAT GGGTCCTCCT     362

GTAAAGCTCT TCACTCCATA AGGTGAAATA GACCTGAAAT ATATATAGAT TATTT          417
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 137:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:

```
 AG ATC ACC ACT GGC ATG CCA GCC TCA ACT GAG ACA GCG TAT GTG TCT       47
Glu Ile Thr Thr Gly Met Pro Ala Ser Thr Glu Thr Ala Tyr Val Ser
 1               5                  10                  15

TCA GAG TCT CCC ATT AGA ATA TCA GTA TCA ACA GAA GGA ACA AAT ACT       95
Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Thr Asn Thr
            20                  25                  30

TCT TCA T                                                            102
Ser Ser Ser
        35
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 138:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:

```
AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT GTG CCC       48
Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro
 1               5                  10                  15

ATG AAA GTC CAA ACC CAA GAA                                           69
Met Lys Val Gln Thr Gln Glu
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 139:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:

```
AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG       48
Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met
 1               5                  10                  15

GCC AGC TTC TAC                                                       60
Ala Ser Phe Tyr
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 140:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:

```
AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA TAG            36
Ser Thr Ser Thr Pro Phe Leu Ser Leu Pr  Glu
  1             5                   10
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 141:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:

```
AAG CAT CTT GGG ATT GAA TTT ATG GAG                        27
Lys His Leu Gly Ile Glu Phe Met Glu
  1             5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 142:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 569
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

```
AAA GCG GAG GAG CTC TAC CAG AAG AGA GTG CTC ACC ATT ACC GGC ATT      48
Lys Ala Glu Glu Leu Tyr Gln Lys Arg Val Leu Thr Ile Thr Gly Ile
  1               5                  10                  15

TGC ATC GCG CTG CTC GTG GTT GGC ATC ATG TGT GTG GTG GTC TAC TGC      96
Cys Ile Ala Leu Leu Val Val Gly Ile Met Cys Val Val Val Tyr Cys
            20                  25                  30

AAA ACC AAG AAA CAA CGG AAA AAG CTT CAT GAC CGG CTT CGG CAG AGC     144
Lys Thr Lys Lys Gln Arg Lys Lys Leu His Asp Arg Leu Arg Gln Ser
        35                  40                  45

CTT CGG TCT GAA AGA AAC ACC ATG ATG AAC GTA GCC AAC GGG CCC CAC     192
Leu Arg Ser Glu Arg Asn Thr Met Met Asn Val Ala Asn Gly Pro His
     50                  55                  60

CAC CCC AAT CCG CCC CCC GAG AAC GTG CAG CTG GTG AAT CAA TAC GTA     240
His Pro Asn Pro Pro Pro Glu Asn Val Gln Leu Val Asn Gln Tyr Val
 65                  70                  75                  80

TCT AAA AAT GTC ATC TCT AGC GAG CAT ATT GTT GAG AGA GAG GCG GAG     288
Ser Lys Asn Val Ile Ser Ser Glu His Ile Val Glu Arg Glu Ala Glu
                 85                  90                  95

AGC TCT TTT TCC ACC AGT CAC TAC ACT TCG ACA GCT CAT CAT TCC ACT     336
Ser Ser Phe Ser Thr Ser His Tyr Thr Ser Thr Ala His His Ser Thr
            100                 105                 110

ACT GTC ACT CAG ACT CCC AGT CAC AGC TGG AGC AAT GGA CAC ACT GAA     384
Thr Val Thr Gln Thr Pro Ser His Ser Trp Ser Asn Gly His Thr Glu
        115                 120                 125

AGC ATC ATT TCG GAA AGC CAC TCT GTC ATC GTG ATG TCA TCC GTA GAA     432
Ser Ile Ile Ser Glu Ser His Ser Val Ile Val Met Ser Ser Val Glu
    130                 135                 140
```

AAC AGT AGG CAC AGC AGC CCG ACT GGG GGC CCG AGA GGA CGT CTC AAT 480
Asn Ser Arg His Ser Ser Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn
145 150 155 160

GGC TTG GGA GGC CCT CGT GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA 528
Gly Leu Gly Gly Pro Arg Glu Cys Asn Ser Phe Leu Arg His Ala Arg
165 170 175

GAA ACC CCT GAC TCC TAC CGA GAC TCT CCT CAT AGT G AAAG 569
Glu Thr Pro Asp Ser Tyr Arg Asp Ser Pro His Ser
180 185

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 143:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 735
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:

G TAT GTA TCA GCA ATG ACC ACC CCG GCT CGT ATG TCA CCT GTA GAT 46
Tyr Val Ser Ala Met Thr Thr Pro Ala Arg Met Ser Pro Val Asp
1 5 10 15

TTC CAC ACG CCA AGC TCC CCC AAG TCA CCC CCT TCG GAA ATG TCC CCG 94
Phe His Thr Pro Ser Ser Pro Lys Ser Pro Pro Ser Glu Met Ser Pro
20 25 30

CCC GTG TCC AGC ACG ACG GTC TCC ATG CCC TCC ATG GCG GTC AGT CCC 142
Pro Val Ser Ser Thr Thr Val Ser Met Pro Ser Met Ala Val Ser Pro
35 40 45

TTC GTG GAA GAG GAG AGA CCC CTG CTC CTT GTG ACG CCA CCA CGG CTG 190
Phe Val Glu Glu Glu Arg Pro Leu Leu Leu Val Thr Pro Pro Arg Leu
50 55 60

CGG GAG AAG TAT GAC CAC CAC GCC CAG CAA TTC AAC TCG TTC CAC TGC 238
Arg Glu Lys Tyr Asp His His Ala Gln Gln Phe Asn Ser Phe His Cys
65 70 75

AAC CCC GCG CAT GAG AGC AAC AGC CTG CCC CCC AGC CCC TTG AGG ATA 286
Asn Pro Ala His Glu Ser Asn Ser Leu Pro Pro Ser Pro Leu Arg Ile
80 85 90 95

GTG GAG GAT GAG GAA TAT GAA ACG ACC CAG GAG TAC GAA CCA GCT CAA 334
Val Glu Asp Glu Glu Tyr Glu Thr Thr Gln Glu Tyr Glu Pro Ala Gln
100 105 110

GAG CCG GTT AAG AAA CTC ACC AAC AGC AGC CGG CGG GCC AAA AGA ACC 382
Glu Pro Val Lys Lys Leu Thr Asn Ser Ser Arg Arg Ala Lys Arg Thr
115 120 125

AAG CCC AAT GGT CAC ATT GCC CAC AGG TTG GAA ATG GAC AAC AAC ACA 430
Lys Pro Asn Gly His Ile Ala His Arg Leu Glu Met Asp Asn Asn Thr
130 135 140

GGC GCT GAC AGC AGT AAC TCA GAG AGC GAA ACA GAG GAT GAA AGA GTA 478
Gly Ala Asp Ser Ser Asn Ser Glu Ser Glu Thr Glu Asp Glu Arg Val
145 150 155

GGA GAA GAT ACG CCT TTC CTG GCC ATA CAG AAC CCC CTG GCA GCC AGT 526
Gly Glu Asp Thr Pro Phe Leu Ala Ile Gln Asn Pro Leu Ala Ala Ser
160 165 170 175

CTC GAG GCG GCC CCT GCC TTC CGC CTG GTC GAC AGC AGG ACT AAC CCA 574
Leu Glu Ala Ala Pro Ala Phe Arg Leu Val Asp Ser Arg Thr Asn Pro
180 185 190

ACA GGC GGC TTC TCT CCG CAG GAA GAA TTG CAG GCC AGG CTC TCC GGT 622
Thr Gly Gly Phe Ser Pro Gln Glu Glu Leu Gln Ala Arg Leu Ser Gly
195 200 205

GTA ATC GCT AAC CAA GAC CCT ATC GCT GTC TAAAACCGAA ATACACCCAT 672
Val Ile Ala Asn Gln Asp Pro Ile Ala Val
210 215

AGATTCACCT GTAAAACTTT ATTTTATATA ATAAAGTATT CCACCTTAAA TTAAACAA 730

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 144:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1654
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:

AGTTTCCCCC CCCAACTTGT CGGAACTCTG GGCTCGCGCG CAGGGCAGGA GCGGAGCGGC 60

GGCGGCTGCC CAGGCGATGC GAGCGCGGGC CGGACGGTAA TCGCCTCTCC CTCCTCGGGC 120

TGCGAGCGCG CCGGACCGAG GCAGCGACAG GAGCGGACCG CGGCGGGAAC CGAGGACTCC 180

CCAGCGGCGC GCCAGCAGGA GCCACCCCGC GAGCGTGCGA CCGGGACGGA GCGCCCGCCA 240

GTCCCAGGTG GCCCGGACCG CACGTTGCGT CCCCGCGCTC CCCGCCGGCG ACAGGAGACG 300

CTCCCCCCCA CGCCGCGCGC GCCTCGGCCC GGTCGCTGGC CCGCCTCCAC TCCGGGGACA 360

AACTTTTCCC GAAGCCGATC CCAGCCCTCG GACCCAAACT TGTCGCGCGT CGCCTTCGCC 420

GGGAGCCCGTC CGCGCAGAGC GTGCACTTCT CGGGCGAG ATG TCG GAG CGC AGA 473
Met Ser Glu Arg Arg
1 5

GAA GGC AAA GGC AAG GGG AAG GGC GGC AAG AAG GAC CGA GGC TCC GGG 521
Glu Gly Lys Gly Lys Gly Lys Gly Gly Lys Lys Asp Arg Gly Ser Gly
10 15 20

AAG AAG CCC GTG CCC GCG GCT GGC GGC CCG AGC CCA GCC TTG CCT CCC 569
Lys Lys Pro Val Pro Ala Ala Gly Gly Pro Ser Pro Ala Leu Pro Pro
25 30 35

CGC TTG AAA GAG ATG AAG ATG CAG GAG TCT GTG GCA GGT TCC AAA CTA 617
Arg Leu Lys Glu Met Lys Ser Gln Glu Ser Val Ala Gly Ser Lys Leu
40 45 50

GTG CTT CGG TGC GAG ACC AGT TCT GAA TAC TCC TCT CTC AAG TTC AAG 665
Val Leu Arg Cys Glu Thr Ser Ser Glu Tyr Ser Ser Leu Lys Phe Lys
55 60 65

TGG TTC AAG AAT GGG AGT GAA TTA AGC CGA AAG AAC AAA CCA CAA AAC 713
Trp Phe Lys Asn Gly Ser Glu Leu Ser Arg Lys Asn Lys Pro Gln Asn
70 75 80 85

ATC AAG ATA CAG AAA AGG CCG GGG AAG TCA GAA CTT CGC ATT AGC AAA 761
Ile Lys Ile Gln Lys Arg Pro Gly Lys Ser Glu Leu Arg Ile Ser Lys
90 95 100

```
GCG TCA CTG GCT GAT TCT GGA GAA TAT ATG TGC AAA GTG ATC AGC AAA     809
Ala Ser Leu Ala Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys
            105                 110                 115

CTA GGA AAT GAC AGT GCC TCT GCC AAC ATC ACC ATT GTG GAG TCA AAC     857
Leu Gly Asn Asp Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn
        120                 125                 130

GAG ATC ACC ACT GGC ATG CCA GCC TCA ACT GAG ACA GCG TAT GTG TCT     905
Glu Ile Thr Thr Gly Met Pro Ala Ser Thr Glu Thr Ala Tyr Val Ser
    135                 140                 145

TCA GAG TCT CCC ATT AGA ATA TCA GTA TCA ACA GAA GGA ACA AAT ACT     953
Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Thr Asn Thr
150                 155                 160                 165

TCT TCA TCC ACA TCC ACA TCT ACA GCT GGG ACA AGC CAT CTT GTC AAG    1001
Ser Ser Ser Thr Ser Thr Ser Thr Ala Gly Thr Ser His Leu Val Lys
                170                 175                 180

TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGC GAG TGC TTC    1049
Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys Phe
            185                 190                 195

ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC TTG TGC AAG TGC CCA    1097
Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro
        200                 205                 210

AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC    1145
Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe
    215                 220                 225

TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT GAA TAGGCGCATG         1191
Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro Glu
230                 235                 240

CTCAGTCGGT GCCGCTTTCT TGTTGCCGCA TCTCCCCTCA GATTCAACCT AGAGCTAGAT  1251

GCGTTTTACC AGGTCTAACA TTGACTGCCT CTGCCTGTCG CATGAGAACA TTAACACAAG  1311

CGATTGTATG ACTTCCTCTG TCCGTGACTA GTGGGCTCTG AGCTACTCGT AGGTGCGTAA  1371

GGCTCCAGTG TTTCTGAAAT TGATCTTGAA TTACTGTGAT ACGACATGAT AGTCCCTCTC  1431

ACCCAGTGCA ATGACAATAA AGGCCTTGAA AAGTCTCACT TTTATTGAGA AAATAAAAAT  1491

CGTTCCACGG GACAGTCCCT CTTCTTTATA AAATGACCCT ATCCTTGAAA AGGAGGTGTG  1551

TTAAGTTGTA ACCAGTACAC ACTTGAAATG ATGGTAAGTT CGCTTCGGTT CAGAATGTGT  1611

TCTTTCTGAC AAATAAACAG AATAAAAAAA AAAAAAAAAA A                      1652
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 145:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1140
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:

```
CAT CAN GTG TGG GCG GCG AAA GCC GGG GGC TTG AAG AAG GAC TCG CTG      48
His Gln Val Trp Ala Ala Lys Ala Gly Gly Leu Lys Lys Asp Ser Leu
1               5                   10                  15

CTC ACC GTG CGC CTG GGC GCC TGG GGC CAC CCC GCC TTC CCC TCC TGC      96
Leu Thr Val Arg Leu Gly Ala Trp Gly His Pro Ala Phe Pro Ser Cys
            20                  25                  30

GGG CGC CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC ATG GAG CCC GAG     144
Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe Met Glu Pro Glu
        35                  40                  45

GCC AAC AGC AGC GGC GGG CCC GGC CGC CTT CCG AGC CTC CTT CCC CCC     192
Ala Asn Ser Ser Gly Gly Pro Gly Arg Leu Pro Ser Leu Leu Pro Pro
    50                  55                  60

TCT CGA GAC GGG CCG GAA CCT CAA GAA GGA GGT CAG CCG GGT GCT GTG     240
Ser Arg Asp Gly Pro Glu Pro Gln Glu Gly Gly Gln Pro Gly Ala Val
65                  70                  75                  80

CAA CGG TGC GCC TTG CCT CCC CGC TTG AAA GAG ATG AAG AGT CAG GAG     288
Gln Arg Cys Ala Leu Pro Pro Arg Leu Lys Glu Met Lys Ser Gln Glu
                85                  90                  95

TCT GTG GCA GGT TCC AAA CTA GTG CTT CGG TGC GAG ACC AGT TCT GAA     336
Ser Val Ala Gly Ser Lys Leu Val Leu Arg Cys Glu Thr Ser Ser Glu
            100                 105                 110

TAC TCC TCT CTC AAG TTC AAG TGG TTC AAG AAT GGG AGT GAA TTA AGC     384
Tyr Ser Ser Leu Lys Phe Lys Trp Phe Lys Asn Gly Ser Glu Leu Ser
        115                 120                 125

CGA AAG AAC AAA CCA GAA AAC ATC AAG ATA CAG AAA AGG CCG GGG AAG     432
Arg Lys Asn Lys Pro Glu Asn Ile Lys Ile Gln Lys Arg Pro Gly Lys
    130                 135                 140

TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA TAT     480
Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu Tyr
145                 150                 155                 160

ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC AAC     528
Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala Asn
                165                 170                 175

ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG ACA     576
Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly Thr
            180                 185                 190

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT     624
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
        195                 200                 205

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC     672
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
    210                 215                 220

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT     720
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
225                 230                 235                 240

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT     768
Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr
                245                 250                 255

GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC     816
 ly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser
            260                 265                 270
```

```
ACT CCC TTT CTG TCT CTG CCT GAA TAGCGCATCT CAGTCGGTGC CGCTTTCTTG     870
Thr Pro Phe Leu Ser Leu Pro Glu
        275                 280

TTGCCGCATC TCCCCTCAGA TTCCNCCTAG AGCTAGATCC GTTTTACCAG GTCTAACATT    930

GACTGCCTCT GCCTGTCGCA TGAGAACATT AACACAAGCG ATTGTATGAC TTCCTCTGTC    990

CGTGACTAGT GGGCTCTGAG CTACTCGTAG GTGCGTAAGG CTCCAGTGTT TCTGAAATTG   1050

ATCTTGAATT ACTGTGATAC GACATGATAG TCCCTCTCAC CCAGTGCAAT GACAATAAAG   1110

GCCTTGAAAA GTCAAAAAAA AAAAAAAAAA                                    1140
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 146:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1764
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:

```
G AAG TCA GAA CTT CGC ATT AGC AAA GCG TCA CTG GCT GAT TCT GGA GAA     49
  Lys Ser Glu Leu Arg Ile Ser Lys Ala Ser Leu Ala Asp Ser Gly Glu
  1               5                   10                  15

TAT ATG TGC AAA GTG ATC AGC AAA CTA GGA AAT GAC AGT GCC TCT GCC       97
Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp Ser Ala Ser Ala
            20                  25                  30

AAC ATC ACC ATT GTG GAG TCA AAC GCC ACA TCC ACA TCT ACA GCT GGG      145
Asn Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr Ser Thr Ala Gly
        35                  40                  45

ACA AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG      193
Thr Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val
    50                  55                  60

AAT GGA GGC GAC TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA      241
Asn Gly Gly Asp Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg
65                  70                  75                  80

TAC TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG      289
Tyr Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu
                85                  90                  95

AAT GTG CCC ATG AAA GTC CAA ACC CAA GAA AAA GCG GAG GAG CTC TAC      337
Asn Val Pro Met Lys Val Gln Thr Gln Glu Lys Ala Glu Glu Leu Tyr
            100                 105                 110

CAG AAG AGA GTG CTC ACC ATT ACC GGC ATT TGC ATC GCG CTG CTC GTG      385
Gln Lys Arg Val Leu Thr Ile Thr Gly Ile Cys Ile Ala Leu Leu Val
        115                 120                 125

GTT GGC ATC ATG TGT GTG GTG GTC TAC TGC AAA ACC AAG AAA CAA CGG      433
Val Gly Ile Met Cys Val Val Val Tyr Cys Lys Thr Lys Lys Gln Arg
    130                 135                 140

AAA AAG CTT CAT GAC CGG CTT CGG CAG AGC CTT CGG TCT GAA AGA AAC      481
Lys Lys Leu His Asp Arg Leu Arg Gln Ser Leu Arg Ser Glu Arg Asn
145                 150                 155                 160
```

```
ACC ATG ATG AAC GTA GCC AAC GGG CCC CAC CAC CCC AAT CCG CCC CCC      529
Thr Met Met Asn Val Ala Asn Gly Pro His His Pro Asn Pro Pro Pro
                165                 170                 175

GAG AAC GTG CAG CTG GTG AAT CAA TAC GTA TCT AAA AAT GTC ATC TCT      577
Glu Asn Val Gln Leu Val Asn Gln Tyr Val Ser Lys Asn Val Ile Ser
            180                 185                 190

AGC GAG CAT ATT GTT GAG AGA GAG GCG GAG AGC TCT TTT TCC ACC AGT      625
Ser Glu His Ile Val Glu Arg Glu Ala Glu Ser Ser Phe Ser Thr Ser
        195                 200                 205

CAC TAC ACT TCG ACA GCT CAT CAT TCC ACT ACT GTC ACT CAG ACT CCC      673
His Tyr Thr Ser Thr Ala His His Ser Thr Thr Val Thr Gln Thr Pro
    210                 215                 220

AGT CAC AGC TGG AGC AAT GGA CAC ACT GAA AGC ATC ATT TCG GAA AGC      721
Ser His Ser Trp Ser Asn Gly His Thr Glu Ser Ile Ile Ser Glu Ser
225                 230                 235                 240

CAC TCT GTC ATC GTG ATG TCA TCC GTA GAA AAC AGT AGG CAC AGC AGC      769
His Ser Val Ile Val Met Ser Ser Val Glu Asn Ser Arg His Ser Ser
                245                 250                 255

CCG ACT GGG GGC CCG AGA GGA CGT CTC AAT GGC TTG GGA GGC CCT CGT      817
Pro Thr Gly Gly Pro Arg Gly Arg Leu Asn Gly Leu Gly Gly Pro Arg
            260                 265                 270

GAA TGT AAC AGC TTC CTC AGG CAT GCC AGA GAA ACC CCT GAC TCC TAC      865
Glu Cys Asn Ser Phe Leu Arg His Ala Arg Glu Thr Pro Asp Ser Tyr
        275                 280                 285

CGA GAC TCT CCT CAT AGT GAA AGA CAT AAC CTT ATA GCT GAG CTA AGG      913
Arg Asp Ser Pro His Ser Glu Arg His Asn Leu Ile Ala Glu Leu Arg
    290                 295                 300

AGA AAC AAG GCC CAC AGA TCC AAA TGC ATG CAG ATC CAG CTT TCC GCA      961
Arg Asn Lys Ala His Arg Ser Lys Cys Met Gln Ile Gln Leu Ser Ala
305                 310                 315                 320

ACT CAT CTT AGA GCT TCT TCC ATT CCC CAT TGG GCT TCA TTC TCT AAG     1009
Thr His Leu Arg Ala Ser Ser Ile Pro His Trp Ala Ser Phe Ser Lys
                325                 330                 335

ACC CCT TGG CCT TTA GGA AGG TAT GTA TCA GCA ATG ACC ACC CCG GCT     1057
Thr Pro Trp Pro Leu Gly Arg Tyr Val Ser Ala Met Thr Thr Pro Ala
            340                 345                 350

CGT ATG TCA CCT GTA GAT TTC CAC ACG CCA AGC TCC CCC AAG TCA CCC     1105
Arg Met Ser Pro Val Asp Phe His Thr Pro Ser Ser Pro Lys Ser Pro
        355                 360                 365

CCT TCG GAA ATG TCC CCG CCC GTG TCC AGC ACG ACG GTC TCC ATG CCC     1153
Pro Ser Glu Met Ser Pro Pro Val Ser Ser Thr Thr Val Ser Met Pro
    370                 375                 380

TCC ATG GCG GTC AGT CCC TTC GTG GAA GAG GAG AGA CCC CTG CTC CTT     1201
Ser Met Ala Val Ser Pro Phe Val Glu Glu Glu Arg Pro Leu Leu Leu
385                 390                 395                 400

GTG ACG CCA CCA CGG CTG CGG GAG AAG TAT GAC CAC CAC GCC CAG CAA     1249
Val Thr Pro Pro Arg Leu Arg Glu Lys Tyr Asp His His Ala Gln Gln
                405                 410                 415

TTC AAC TCG TTC CAC TGC AAC CCC GCG CAT GAG AGC AAC AGC CTG CCC     1297
Phe Asn Ser Phe His Cys Asn Pro Ala His Glu Ser Asn Ser Leu Pro
            420                 425                 430
```

```
CCC AGC CCC TTG AGG ATA GTG GAG GAT GAG GAA TAT GAA ACG ACC CAG        1345
Pro Ser Pro Leu Arg Ile Val Glu Asp Glu Glu Tyr Glu Thr Thr Gln
        435                 440                 445

GAG TAC GAA CCA GCT CAA GAG CCG GTT AAG AAA CTC ACC AAC AGC AGC        1393
Glu Tyr Glu Pro Ala Gln Glu Pro Val Lys Lys Leu Thr Asn Ser Ser
    450                 455                 460

CGG CGG GCC AAA AGA ACC AAG CCC AAT GGT CAC ATT GCC CAC AGG TTG        1441
Arg Arg Ala Lys Arg Thr Lys Pro Asn Gly His Ile Ala His Arg Leu
465                 470                 475                 480

GAA ATG GAC AAC AAC ACA GGC GCT GAC AGC AGT AAC TCA GAG AGC GAA        1489
Glu Met Asp Asn Asn Thr Gly Ala Asp Ser Ser Asn Ser Glu Ser Glu
                485                 490                 495

ACA GAG GAT GAA AGA GTA GGA GAA GAT ACG CCT TTC CTG GCC ATA CAG        1537
Thr Glu Asp Glu Arg Val Gly Glu Asp Thr Pro Phe Leu Ala Ile Gln
            500                 505                 510

AAC CCC CTG GCA GCC AGT CTC GAG GCG GCC CCT GCC TTC CGC CTG GTC        1585
Asn Pro Leu Ala Ala Ser Leu Glu Ala Ala Pro Ala Phe Arg Leu Val
        515                 520                 525

GAC AGC AGG ACT AAC CCA ACA GGC GGC TTC TCT CCG CAG GAA GAA TTG        1633
Asp Ser Arg Thr Asn Pro Thr Gly Gly Phe Ser Pro Gln Glu Glu Leu
    530                 535                 540

CAG GCC AGG CTC TCC GGT GTA ATC GCT AAC CAA GAC CCT ATC GCT GTC        1681
Gln Ala Arg Leu Ser Gly Val Ile Ala Asn Gln Asp Pro Ile Ala Val
545                 550                 555                 560

TAAAACCGAA ATACACCCAT AGATTCACCT GTAAAACTTT ATTTTATATA ATAAAGTATT      1741

CCACCTTAAA TTAAACAAAA AAA                                              1764
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 147:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:

```
Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
1               5                   10                  15

Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys
            20                  25                  30

Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser
        35                  40                  45

Phe Tyr
    50
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 148:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 50
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTI N: SEQ ID NO: 148:

Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys
1 5 10 15

Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu Cys Lys Cys
20 25 30

Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn Val Pro Met Lys
35 40 45

Val Gln
50

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 149:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

Glu Cys Leu Arg Lys Tyr Lys Asp Phe Cys Ile His Gly Glu Cys Lys
1 5 10 15

Tyr Val Lys Glu Leu Arg Ala Pro Ser Cys Lys Cys Gln Gln Glu Tyr
20 25 30

Phe Gly Glu Arg Cys Gly Glu Lys Ser Asn Lys Thr His Ser
35 40 45

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 150:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 198
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT 48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
1 5 10 15

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC 96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
20 25 30

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC 144
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr
35 40 45

GTA ATG GCC AGC TTC TAC AGT ACG TCC ACT CCC TTT CTG TCT CTG CCT 192
Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro Phe Leu Ser Leu Pro
50 55 60

GAA TAG 198
Glu
65

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 151:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 192
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT 48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
1 5 10 15

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC 96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
20 25 30

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT 144
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
35 40 45

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAA GCG GAG GAG CTC TAC TAA 192
Val Pro Met Lys Val Gln Thr Gln Glu Lys Ala Glu Glu Leu Tyr
50 55 60

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 152:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 183
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT 48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
1 5 10 15

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC 96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
20 25 30

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC 144
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr
35 40 45

GTA ATG GCC AGC TTC TAC AAA GCG GAG GAG CTC TAC TAA 183
Val Met Ala Ser Phe Tyr Lys Ala Glu Glu Leu Tyr
50 55 60

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 153:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

```
AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT      48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
1               5                   10                  15

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC      96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
            20                  25                  30

TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC     144
Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp Arg Cys Gln Asn Tyr
        35                  40                  45

GTA ATG GCC AGC TTC TAC AAG CAT CTT GGG ATT GAA TTT ATG GAG AAA     192
Val Met Ala Ser Phe Tyr Lys His Leu Gly Ile Glu Phe Met Glu Lys
    50                  55                  60

GCG GAG GAG CTC TAC TAA                                             210
Ala Glu Glu Leu Tyr
65
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 154:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 267
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

```
AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT      48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
1               5                   10                  15

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC      96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
            20                  25                  30

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT     144
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
        35                  40                  45

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT     192
Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr
    50                  55                  60

GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AGT ACG TCC     240
Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser
65                  70                  75                  80

ACT CCC TTT CTG TCT CTG CCT GAA TAG                                 267
Thr Pro Phe Leu Ser Leu Pro Glu
                85
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 155:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 252

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:

```
AGC CAT CTT GTC AAG TGT GCA GAG AAG GAG AAA ACT TTC TGT GTG AAT      48
Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys Thr Phe Cys Val Asn
1               5                   10                  15

GGA GGC GAG TGC TTC ATG GTG AAA GAC CTT TCA AAT CCC TCA AGA TAC      96
Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser Asn Pro Ser Arg Tyr
            20                  25                  30

TTG TGC AAG TGC CAA CCT GGA TTC ACT GGA GCG AGA TGT ACT GAG AAT     144
Leu Cys Lys Cys Gln Pro Gly Phe Thr Gly Ala Arg Cys Thr Glu Asn
        35                  40                  45

GTG CCC ATG AAA GTC CAA ACC CAA GAA AAG TGC CCA AAT GAG TTT ACT     192
Val Pro Met Lys Val Gln Thr Gln Glu Lys Cys Pro Asn Glu Phe Thr
    50                  55                  60

GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AAA GCG GAG     240
Gly Asp Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Lys Ala Glu
65                  70                  75                  80

GAG CTC TAC TAA                                                     252
Glu Leu Tyr
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 156:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 128
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 156:

```
CC ACA TCC ACA TCT ACA GCT GGG ACA AGC CAT CTT GTC AAG TGT GCA       47
   Thr Ser Thr Ser Thr Ala Gly Thr Ser His Leu Val Lys Cys Ala
   1               5                   10                  15

GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGC GAG TGC TTC ATG GTG      95
Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Cys Phe Met Val
            20                  25                  30

AAA GAC CTT TCA AAT CCC TCA AGA TAC TTG T GC                        128
Lys Asp Leu Ser Asn Pro Ser Arg Tyr Leu
        35                  40
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 157:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 141
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:

```
A CAT AAC CTT ATA GCT GAG CTA AGG AGA AAC AAG GCC CAC AGA TCC      46
  His Asn Leu Ile Ala Glu Leu Arg Arg Asn Lys Ala His Arg Ser
   1               5                  10                  15

AAA TGC ATG CAG ATC CAG CTT TCC GCA ACT CAT CTT AGA GCT TCT TCC    94
Lys Cys Met Gln Ile Gln Leu Ser Ala Thr His Leu Arg Ala Ser Ser
                20                  25                  30

ATT CCC CAT TGG GCT TCA TTC TCT AAG ACC CCT TGG CCT TTA GGA AG    141
Ile Pro His Trp Ala Ser Phe Ser Lys Thr Pro Trp Pro Leu Gly Arg
            35                  40                  45
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 158:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in positions 15 and 22 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:

```
Ala Ala Glu Lys Glu Lys Thr Phe Cys Val Asn Gly Gly Glu Xaa Phe
 1               5                  10                  15

Met Val Lys Asp Leu Xaa Asn Pro
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 159:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 745
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:

```
ATG AGA TGG CGA CGC GCC CCG CGC CGC TCC GGG CGT CCC GGC CCC CGG    48
Met Arg Trp Arg Arg Ala Pro Arg Arg Ser Gly Arg Pro Gly Pro Arg
1               5                   10                  15

GCC CAG CGC CCC GGC TCC GCC GCC CGC TCG TCG CCG CCG CTG CCG CTG    96
Ala Gln Arg Pro Gly Ser Ala Ala Arg Ser Ser Pro Pro Leu Pro Leu
            20                  25                  30

CTG CCA CTA CTG CTG CTG CTG GGG ACC GCG GCC CTG GCG CCG GGG GCG   144
Leu Pro Leu Leu Leu Leu Leu Gly Thr Ala Ala Leu Ala Pro Gly Ala
        35                  40                  45

GCG GCC GGC AAC GAG GCG GCT CCC GCG GGG GCC TCG GTG TGC TAC TCG   192
Ala Ala Gly Asn Glu Ala Ala Pro Ala Gly Ala Ser Val Cys Tyr Ser
    50                  55                  60

TCC CCG CCC AGC GTG GGA TCG GTG CAG GAG CTA GCT CAG CGC GCC GCG   240
Ser Pro Pro Ser Val Gly Ser Val Gln Glu Leu Ala Gln Arg Ala Ala
65                  70                  75                  80
```

GTG GTG ATC GAG GGA AAG GTG CAC CCG CAG CGG CGG CAG CAG GGG GCA 288
Val Val Ile Glu Gly Lys Val His Pro Gln Arg Arg Gln Gln Gly Ala
85 90 95

CTC GAC AGG AAG GCG GCG GCG GCG GCG GGC GAG GCA GGG GCG TGG GGC 336
Leu Asp Arg Lys Ala Ala Ala Ala Ala Gly Glu Ala Gly Ala Trp Gly
100 105 110

GGC GAT CGC GAG CCG CCA GCC GCG GGC CCA CGG GCG CTG GGG CCG CCC 384
Gly Asp Arg Glu Pro Pro Ala Ala Gly Pro Arg Ala Leu Gly Pro Pro
115 120 125

GCC GAG GAG CCG CTG CTC GCC GCC AAC GGG ACC GTG CCC TCT TGG CCC 432
Ala Glu Glu Pro Leu Leu Ala Ala Asn Gly Thr Val Pro Ser Trp Pro
130 135 140

ACC GCC CCG GTG CCC AGC GCC GGC GAG CCC GGG GAG GAG GCG CCC TAT 480
Thr Ala Pro Val Pro Ser Ala Gly Glu Pro Gly Glu Glu Ala Pro Tyr
145 150 155 160

CTG GTG AAG GTG CAC CAG GTG TGG GCG GTG AAA GCC GGG GGC TTG AAG 528
Leu Val Lys Val His Gln Val Trp Ala Val Lys Ala Gly Gly Leu Lys
165 170 175

AAG GAC TCG CTG CTC ACC GTG CGC CTG GGG ACC TGG GGC CAC CCC GCC 576
Lys Asp Ser Leu Leu Thr Val Arg Leu Gly Thr Trp Gly His Pro Ala
180 185 190

TTC CCC TCC TGC GGG AGG CTC AAG GAG GAC AGC AGG TAC ATC TTC TTC 624
Phe Pro Ser Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe
195 200 205

ATG GAG CCC GAC GCC AAC AGC ACC AGC CGC GCG CCG GCC GCC TTC CGA 672
Met Glu Pro Asp Ala Asn Ser Thr Ser Arg Ala Pro Ala Ala Phe Arg
210 215 220

GCC TCT TTC CCC CCT CTG GAG ACG GGC CGG AAC CTC AAG AAG GAG GTC 720
Ala Ser Phe Pro Pro Leu Glu Thr Gly Arg Asn Leu Lys Lys Glu Val
225 230 235 240

AGC CGG GTG CTG TGC AAG CGG TGC G 745
Ser Arg Val Leu Cys Lys Arg Cys
245

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 160:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:

Xaa Ala Leu Ala Ala Ala Gly Tyr Asp Val Glu Lys
1 5 10

(2) INFORMATION F R SEQUENCE IDENTIFICATION NUMBER: 161:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in position 1 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161:

Xaa Leu Val Leu Arg
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 162:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: Xaa in positions 1, 2, and 3 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:

Xaa Xaa Xaa Tyr Pro Gly Gln Ile Thr Ser Asn
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 163:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N in positions 25 and 36 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 163:

ATAGGGAAGG GCGGGGGAAG GGTCNCCCTC NGCACGGCCG GGCTTGCCTC TGGAGCCTCT 60

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 164:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: N in position 16 is unknown.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 164:

TTTACACATA TATTCNCC 18

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 165:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 165:

```
Glu Thr Gln Pro Asp Pro Gly Gln Ile Leu Lys Lys Val Pro Met Val
 1               5                  10                  15

Ile Gly Ala Tyr Thr
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 166:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 422
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 166:

```
Met Arg Trp Arg Arg Ala Pro Arg Arg Ser Gly Arg Pro Gly Pro Arg
 1               5                  10                  15

Ala Gln Arg Pro Gly Ser Ala Ala Arg Ser Ser Pro Pro Leu Pro Leu
            20                  25                  30

Leu Pro Leu Leu Leu Leu Leu Gly Thr Ala Ala Leu Ala Pro Gly Ala
        35                  40                  45

Ala Ala Gly Asn Glu Ala Ala Pro Ala Gly Ala Ser Val Cys Tyr Ser
    50                  55                  60

Ser Pro Pro Ser Val Gly Ser Val Gln Glu Leu Ala Gln Arg Ala Ala
65                  70                  75                  80

Val Val Ile Glu Gly Lys Val His Pro Gln Arg Arg Gln Gln Gly Ala
                85                  90                  95

Leu Asp Arg Lys Ala Ala Ala Ala Ala Gly Glu Ala Gly Ala Trp Gly
            100                 105                 110

Gly Asp Arg Glu Pro Pro Ala Ala Gly Pro Arg Ala Leu Gly Pro Pro
        115                 120                 125

Ala Glu Glu Pro Leu Leu Ala Ala Asn Gly Thr Val Pro Ser Trp Pro
    130                 135                 140

Thr Ala Pro Val Pro Ser Ala Gly Glu Pro Gly Glu Glu Ala Pro Tyr
145                 150                 155                 160
```

Leu Val Lys Val His Gln Val Trp Ala Val Lys Ala Gly Gly Leu Lys
165 170 175

Lys Asp Ser Leu Leu Thr Val Arg Leu Gly Thr Trp Gly His Pro Ala
180 185 190

Phe Pro Ser Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe
195 200 205

Met Glu Pro Asp Ala Asn Ser Thr Ser Arg Ala Pro Ala Ala Phe Arg
210 215 220

Ala Ser Phe Pro Pro Leu Glu Thr Gly Arg Asn Leu Lys Lys Glu Val
225 230 235 240

Ser Arg Val Leu Cys Lys Arg Cys Ala Leu Pro Pro Gln Leu Lys Glu
245 250 255

Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys
260 265 270

Glu Thr Ser Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn
275 280 285

Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln
290 295 300

Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
305 310 315 320

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp
325 330 335

Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr
340 345 350

Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys
355 360 365

Thr Phe Cys Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser
370 375 380

Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp
385 390 395 400

Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pro
405 410 415

Phe Leu Ser Leu Pro Glu
420

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 167:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 167:

Met Ser Glu Arg Lys Glu Gly Arg Gly Lys Gly Lys Gly Lys Lys Lys
1 5 10 15

```
Glu Arg Gly Ser Gly Lys Lys Pr  Glu Ser Ala Ala Gly Ser Gln Ser
            20                  25                  30

Pro Arg Glu Ile Ile Thr Gly Met Pro Ala Ser Thr Glu Gly Ala Tyr
        35                  40                  45

Val Ser Ser Glu Ser Pro Ile Arg Ile Ser Val Ser Thr Glu Gly Ala
    50                  55                  60

Asn Thr Ser Ser Ser
65
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 168:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 168:

```
Arg Lys Gly Asp Val Pro Gly Pro Arg Val Lys Ser Ser Arg Ser Thr
1               5                   10                  15

Thr Thr Ala
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 169:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 231
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:

```
CGCGAGCGCC TCAGCGCGGC CGCTCGCTCT CCCCCTCGAG GGACAAACTT TTCCCAAACC    60

CGATCCGAGC CCTTGGACCA AACTCGCCTG CGCCGAGAGC CGTCCGCGTA GAGCGCTCCG   120

TCTCCGGCGA GATGTCCGAG CGCAAAGAAG GCAGAGGCAA AGGGAAGGGC AAGAAGAAGG   180

AGCGAGGCTC CGGCAAGAAG CCGGAGTCCG CGGCGGGCAG CCAGAGCCCA C            231
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 170:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 178
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:

```
CCTTGCCTCC CCGATTGAAA GAGATGAAAA GCCAGGAATC GGCTGCAGGT TCCAAACTAG    60

TCCTTCGGTG TGAAACCAGT TCTGAATACT CCTCTCTCAG ATTCAAGTGG TTCAAGAATG   120

CGAATGAATT GAATCGAAAA AACAAACCAC AAAATATCAA GATACAAAAA AAGCCAGG     178
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 171:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:

GAAGTCAGAA CTTCGCATTA ACAAAGCATC ACTGGCTGAT TCTGGAGAGT ATATGTCCAA 60

AGTGATCAGC AAATTAGGAA ATGACAGTGC CTCTGCCAAT ATCACCATCG TGGAATCAAA 120

CG 122

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 172:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 172:

AGATCATCAC TGGTATGCCA GCCTCAACTG AAGGAGCATA TGTGTCTTCA GAGTCTCCCA 60

TTAGAATATC AGTATCCACA GAAGGAGCAA ATACTTCTTC AT 102

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 173:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 128
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 173:

CTACATCTAC ATCCACCACT GGGACAAGCC ATCTTGTAAA ATGTGCCGAG AAGGAGAAAA 60

CTTTCTGTGT GAATGGAGGG GAGTGCTTCA TGGTGAAAGA CCTTTCAAAC CCCTCGAGAT 120

ACTTGTGC 128

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 174:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 69
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 174:

AAGTGCCAAC CTGGATTCAC TGGAGCAAGA TGTACTGAGA ATGTGCCCAT GAAAGTCCAA 60

AACCAAGAA 69

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 175:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 175:

AAGTGCCCAA ATGAGTTTAC TGGTGATCGC TGCCAAAACT ACGTAATGGC CAGCTTCTAC 60

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 176:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 176:

AGTACGTCCA CTCCCTTTCT GTCTCTGCCT GAATAG 36

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 177:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 569
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 177:

AAGGCGGAGG AGCTGTACCA GAAGAGAGTG CTGACCATAA CCGGCATCTG CATCGCCCTC 60

CTTGTGGTCG GCATCATGTG TGTGGTGGCC TACTGCAAAA CCAAGAAACA GCGGAAAAAG 120

CTGCATGACC GTCTTCGGCA GAGCCTTCGG TCTGAACGAA ACAATATGAT GAACATTGCC 180

AATGGGCCTC ACCATCCTAA CCCACCCCCC GAGAATGTCC AGCTGGTGAA TCAATACGTA 240

TCTAAAAACG TCATCTCCAG TGAGCATATT GTTGAGAGAG AAGCAGAGAC ATCCTTTTCC 300

ACCAGTCACT ATACTTCCAC AGCCCATCAC TCCACTACTG TCACCCAGAC TCCTAGCCAC 360

AGCTGGAGCA ACGGACACAC TGAAAGCATC CTTTCCGAAA GCCACTCTGT AATCGTGATG 420

TCATCCGTAG AAAACAGTAG GCACAGCAGC CCAACTGGGG GCCCAAGAGG ACGTCTTAAT 480

GGCACAGGAG GCCCTCGTGA ATGTAACAGC TTCCTCAGGC ATGCCAGAGA AACCCCTGAT 540

TCCTACCGAG ACTCTCCTCA TAGTGAAAG 569

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 178:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 730
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 178:

GTATGTGTCA GCCATGACCA CCCCGGCTCG TATGTCACCT GTAGATTTCC ACACGCCAAG 60
CTCCCCCAAA TCGCCCCCTT CGGAAATGTC TCCACCCGTG TCCAGCATGA CGGTGTCCAT 120
GCCTTCCATG GCGGTCAGCC CCTTCATGGA AGAAGAGAGA CCTCTACTTC TCGTGACACC 180
ACCAAGGCTG CGGGAGAAGA AGTTTGACCA TCACCCTCAG CAGTTCAGCT CCTTCCACCA 240
CAACCCCGCG CATGACAGTA ACAGCCTCCC TGCTAGCCCC TTGAGGATAG TGGAGGATGA 300
GGAGTATGAA ACGACCCAAG AGTACGAGCC AGCCCAAGAG CCTGTTAAGA AACTCGCCAA 360
TAGCCGGCGG GCCAAAAGAA CCAAGCCCAA TGGCCACATT GCTAACAGAT TGGAAGTGGA 420
CAGCAACACA AGCTCCCAGA GCAGTAACTC AGAGAGTGAA ACAGAAGATG AAAGAGTAGG 480
TGAAGATACG CCTTTCCTGG GCATACAGAA CCCCCTGGCA GCCAGTCTTG AGGCAACACC 540
TGCCTTCCGC CTGGCTGACA GCAGGACTAA CCCAGCAGGC CGCTTCTCGA CACAGGAAGA 600
AATCCAGGCC AGGCTGTCTA GTGTAATTGC TAACCAAGAC CCTATTGCTG TATAAAACCT 660
AAATAAACAC ATAGATTCAC CTGTAAAACT TTATTTTATA TAATAAAGTA TTCCACCTTA 720
AATTAAACAA 730

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 179:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 179:

TCGGGCTCCA TGAAGAAGAT GTA 23

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 180:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 180:

TCCATGAAGA AGATGTACCT GCT 23

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 181:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 181:

ATGTACCTGC TGTCCTCCTT GA 22

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 182:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 182:

TTGAAGAAGG ACTCGCTGCT CA 22

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 183:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 183:

AAAGCCGGGG GCTTGAAGAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 184:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 184:

ATGARGTGTG GGCGGCGAAA 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 185:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 422
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 185:

```
Met Arg Trp Arg Arg Ala Pro Arg Arg Ser Gly Arg Pro Gly Pro Arg
1               5                   10                  15

Ala Gln Arg Pro Gly Ser Ala Ala Arg Ser Ser Pro Pro Leu Pro Leu
            20                  25                  30

Leu Pro Leu Leu Leu Leu Leu Gly Thr Ala Ala Leu Ala Pr  Gly Ala
        35                  40                  45
```

Ala Ala Gly Asn Glu Ala Ala Pr Ala Gly Ala Ser Val Cys Tyr Ser
50 55 60

Ser Pro Pro Ser Val Gly Ser Val Gln Glu Leu Ala Gln Arg Ala Ala
65 70 75 80

Val Val Ile Glu Gly Lys Val His Pro Gln Arg Arg Gln Gln Gly Ala
85 90 95

Leu Asp Arg Lys Ala Ala Ala Ala Ala Gly Glu Ala Gly Ala Trp Gly
100 105 110

Gly Asp Arg Glu Pro Pro Ala Ala Gly Pro Arg Ala Leu Gly Pro Pro
115 120 125

Ala Glu Glu Pro Leu Leu Ala Ala Asn Gly Thr Val Pro Ser Trp Pro
130 135 140

Thr Ala Pro Val Pro Ser Ala Gly Glu Pro Gly Glu Glu Ala Pro Tyr
145 150 155 160

Leu Val Lys Val His Gln Val Trp Ala Val Lys Ala Gly Gly Leu Lys
165 170 175

Lys Asp Ser Leu Leu Thr Val Arg Leu Gly Thr Trp Gly His Pro Ala
180 185 190

Phe Pro Ser Cys Gly Arg Leu Lys Glu Asp Ser Arg Tyr Ile Phe Phe
195 200 205

Met Glu Pro Asp Ala Asn Ser Thr Ser Arg Ala Pro Ala Ala Phe Arg
210 215 220

Ala Ser Phe Pro Pro Leu Glu Thr Gly Arg Asn Leu Lys Lys Glu Val
225 230 235 240

Ser Arg Val Leu Cys Lys Arg Cys Ala Leu Pro Pro Gln Leu Lys Glu
245 250 255

Met Lys Ser Gln Glu Ser Ala Ala Gly Ser Lys Leu Val Leu Arg Cys
260 265 270

Glu Thr Ser Ser Glu Tyr Ser Ser Leu Arg Phe Lys Trp Phe Lys Asn
275 280 285

Gly Asn Glu Leu Asn Arg Lys Asn Lys Pro Gln Asn Ile Lys Ile Gln
290 295 300

Lys Lys Pro Gly Lys Ser Glu Leu Arg Ile Asn Lys Ala Ser Leu Ala
305 310 315 320

Asp Ser Gly Glu Tyr Met Cys Lys Val Ile Ser Lys Leu Gly Asn Asp
325 330 335

Ser Ala Ser Ala Asn Ile Thr Ile Val Glu Ser Asn Ala Thr Ser Thr
340 345 350

Ser Thr Thr Gly Thr Ser His Leu Val Lys Cys Ala Glu Lys Glu Lys
355 360 365

Thr Phe Cys Val Asn Gly Gly Glu Cys Phe Met Val Lys Asp Leu Ser
370 375 380

Asn Pro Ser Arg Tyr Leu Cys Lys Cys Pro Asn Glu Phe Thr Gly Asp
385 390 395 400

Arg Cys Gln Asn Tyr Val Met Ala Ser Phe Tyr Ser Thr Ser Thr Pr
405 410 415

Phe Leu Ser Leu Pro Glu
420

What is claimed is:

1. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide encoded by pGGF2HBS5 deposited with the A.T.C.C. November 6, 1992 (A.T.C.C. Deposit No. 75347) with a pharmaceutical carrier.

2. A method of making a medicament for the treating of muscle cells of a mammal, said method comprising admixing a polypeptide encoded by the E sequence (SEQ ID Nos. 133 and 159) and at least a portion of the peptide encoded by the DNA sequences flanking the E encoding sequence on clone pGGF2HBS5, deposited with the A.T.C.C. November 6, 1992 (A.T.C.C. Deposit No. 75347).

3. A method of making a medicament for the treating of muscle cells of a mammal, said method comprising admixing a polypeptide defined by the formula

YBAZCX

wherein YBAZCX is composed of the polypeptide segments shown in Fig. 30 (SEQ ID Nos. 133-135, 156, 159); wherein Y comprises polypeptide segment E, or is absent; wherein Z comprises polypeptide segment G or is absent; and wherein X comprises polypeptide segments C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL, or C/D C/D' D' HKL with a pharmaceutical carrier.

4. A method of making a medicament for the treating of muscle cells of a mammal, said method comprising admixing a polypeptide defined by the formula

WBAZCX

wherein WBAZCX is composed of the polypeptide segments shown in Fig. 30 (SEQ ID Nos. 132, 134, 135, 137-139, 156); wherein W comprises polypeptide segment F, or is absent; wherein Z comprises polypeptide segment G or is absent; and wherein X comprises polypeptide segments C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL, or C/D C/D' D' HKL with a pharmaceutical carrier.

5. The method of any one of claims 1-3, wherein 50 N-terminal amino acids are cleaved from said peptide comprising the E sequence (SEQ ID Nos. 133 and 159).

6. The method of claim 3 or 4, wherein X is C/D HKL.

7. The method of claim 3 or 4, wherein X is C/D H.

8. The method of claim 3 or 4, wherein X is C/D HL.

9. The method of claim 3 or 4, wherein X is C/D D.

10. The method of claim 3 or 4, wherein X is C/D' HL.

11. The method of claim 3 or 4, wh rein X is C/D′ HKL.

12. The method of claim 3 or 4, wherein X is C/D′ H.

13. The method of claim 3 or 4, wherein X is C/D′ D.

14. The method of claim 3 or 4, wherein X is C/D C/D′ HKL.

15. The method of claim 3 or 4, wherein X is C/D C/D′ H.

16. The method of claim 3 or 4, wherein X is C/D C/D′ HL.

17. The method of claim 3 or 4, wherein X is C/D C/D′ D.

18. The method of claim 3 or 4, wherein X is C/D D′ H.

19. The method of claim 3 or 4, wherein X is C/D D′ HL.

20. The method of claim 3 or 4, wherein X is C/D D′ HKL.

21. The method of claim 3 or 4, wherein X is C/D′ D′ H.

22. The method of claim 3 or 4, wherein X is C/D' D' HL.

23. The method of claim 3 or 4, wherein X is C/D' D' HKL.

24. The method of claim 3 or 4, wherein X is C/D C/D' D' H.

25. The method of claim 3 or 4, wherein X is C/D C/D' D' HL.

26. The method of claim 3 or 4, wherein X is C/D C/D' D' HKL.

27. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising FBA polypeptide segments having the amino acid sequences shown in Fig. 30 (SEQ ID Nos. 132, 134, 135) with a pharmaceutically acceptable carrier.

28. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising FBA' polypeptide segments having the amino acid sequences shown in Fig. 30 (SEQ ID Nos. 132, 134, 136) with a pharmaceutically acceptable carrier.

29. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising FEBA polypeptide segments having the amino acid sequences shown in Fig. 30 (SEQ ID Nos. 132, 135, 159) with a pharmaceutically acceptable carrier.

30. A meth d of making a m dicament for th treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising FEBA' polypeptide segments having the amino acid sequences corresponding to polypeptide segments shown in Fig. 30 (SEQ ID Nos. 132-134, 136, 159) to muscle cells with a pharmaceutically acceptable carrier.

31. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing GGF2 polypeptide with a pharmaceutically acceptable carrier.

32. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a compound which specifically binds the $p185^{erbB2}$ receptor of muscle cells with a pharmaceutically acceptable carrier.

33. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising EGFL1, having the amino acid sequence shown Fig. 37, Seq. ID No. 150, with a pharmaceutically acceptable carrier.

34. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising EGFL2, having the amino acid sequence shown in Fig. 38, Seq. ID No. 151, with a pharmaceutically acceptable carrier.

35. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising EGFL3, with the amino acid sequence shown in Fig. 39, Seq. ID No. 152, with a pharmaceutically acceptable carrier.

36. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising EGFL4, with the amino acid sequence shown in Fig. 40, Seq. ID No. 153, with a pharmaceutically acceptable carrier.

37. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide comprising EGFL5, with the amino acid sequence shown in Fig. 41, Seq. ID No. 154, to muscle cells, with a pharmaceutically acceptable carrier.

38. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a polypeptide, comprising EGFL6, with the amino acid sequence shown Fig. 42, Seq. ID No. 155, with a pharmaceutically acceptable carrier.

39. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 35 kD polypeptide factor isolated from the rat I-EJ transformed fibroblast cell line to said muscle cells, with a pharmaceutically acceptable carrier.

40. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 75 kD polypeptide factor isolated from the SKBR-3 human breast cell line to said muscle cells, with a pharmaceutically acceptable carrier.

41. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 44 kD polypeptide factor isolated from the rat I-EJ transformed fibroblast cell line to said muscle cells, with a pharmaceutically acceptable carrier.

42. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 45 kD polypeptide factor isolated from the MDA - MB 231 human breast cell line to said muscle cells, with a pharmaceutically acceptable carrier.

43. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 7 to 14 kD polypeptide factor isolated from the ATL-2 human T-cell line to said muscle cells, with a pharmaceutically acceptable carrier.

44. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 25 kD polypeptide factor isolated from activated mouse peritoneal macrophages to said muscle cells, with a pharmaceutically acceptable carrier.

45. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 25 kD polypeptide factor isolated from bovine kidney to said muscle cells, with a pharmaceutically acceptable carrier.

46. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a ARIA polypeptide to said muscle cells, with a pharmaceutically acceptable carrier.

47. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing a 46-47 kD polypeptide factor which stimulates 0-2A glial progenitor cells to said muscle cells, with a pharmaceutically acceptable carrier.

48. A method of making a medicament for the treating of muscle cells of a mammal said method comprising admixing GGF-III to said muscle cells, with a pharmaceutically acceptable carrier.

49. A method of making a medicament for th treating of muscle cells of a mammal, said method comprising admixing with a pharmaceutically acceptable carrier, a DNA sequence encoding a polypeptide of the formula

YBAZCX

wherein YBAZCX is composed of the polypeptide segments shown in Fig. 30 (SEQ ID Nos. 133-135, 156, 159); wherein Y comprises polypeptide segment E, or is absent; wherein Z comprises polypeptide segment G or is absent; and wherein X comprises polypeptide segments C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL, or C/D C/D' D' HKL, said DNA in an expressible genetic construction.

50. A method of making a medicament for the treating of muscle cells of a mammal, said method comprising admixing with a pharmaceutically acceptable carrier, a DNA sequence encoding a polypeptide of the formula

WBAZCX

wherein WBAZCX is composed of the polypeptide segments shown in Fig. 30 (SEQ ID Nos. 132, 134, 135, 137-139, 156); wherein W comprises polypeptide segment F, or is absent; wherein Z comprises polypeptide segment G or is absent; and wherein X comprises polypeptide segments C/D HKL, C/D H, C/D HL, C/D D, C/D' HL, C/D' HKL, C/D' H, C/D' D, C/D C/D' HKL, C/D C/D' H, C/D C/D' HL, C/D C/D' D, C/D D' H, C/D D' HL, C/D D' HKL, C/D' D' H, C/D' D' HL, C/D' D' HKL, C/D C/D' D' H, C/D C/D' D' HL, or C/D C/D' D' HKL, said DNA in an expressible genetic construction.

51. A method of making a medicament for the prophylaxis or treatment of pathophysiological condition of the musculature in a mammal in which said condition involves a muscle cell type which is sensitive or responsive to a polypeptide as defined in any one of claims 1, 3, 4, and 31, said method comprising admixing an effective amount of said polypeptide with a pharmaceutically acceptable carrier.

52. A method of making a medicament for the treatment of a condition which involves muscle damage in a mammal, said method comprising admixing an effective amount of a polypeptide, as defined in any one of claims 1, 3, 4, and 31 with a pharaceutically acceptable carrier.

53. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for decreasing the atrophy of said muscle cells.

54. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for increasing the muscle fibers present in said mammal.

55. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for increasing muscle cell survival in a said mammal.

56. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for increasing muscle growth in a said mammal.

57. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for increasing muscle regeneration in a said mammal.

58. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for stimulating muscle cell mitogenesis.

59. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for increasing acetylcholine receptor synthesis.

60. The method of any one of claims 1, 3, 4, and 31, wherein said medicament is for treating a patient lacking a neurotrophic factor.

61. A method of claims 1, 3, 4, and 31, wherein said medicament is for treating a muscle cell which is a myoblast.

62. A method of claims 1, 3, 4, and 31, wherein said medicament is for treating a muscle cell which is a satellite cell.

63. A method of claims 1, 3, 4, and 31 wherein said medicament is for treating a muscle cell in skeletal muscle.

64. A method of claims 1, 3, 4, and 31 wherein said medicament is for treating a muscle cell in cardiac muscle.

65. A method of claims 1, 3, 4, and 31 wherein said medicament is for treating a muscle cell in smooth muscle.

66. A method of claims 1, 3, 4, and 31, wherein said medicament is for treating a muscle cell in a patient with a skeletal muscle disease.

67. A method of claim 66, wherein said skeletal muscle disease is a myopathy.

68. A method of claim 66, wherein said skeletal muscle disease is a dystrophy.

69. A method of claim 68, wherein said dystrophy is Duchennes muscular dystrophy.

70. A m thod of claim 68, wherein said dystrophy is Beckker's dystrophy.

71. A method of claim 66, wherein said skeletal muscle disease is a result of a neural condition.

72. A method of claim 66, wherein said skeletal muscle disease is an injury.

73. A method of claim 66, wherein said skeletal muscle disease is resulting from a nerve injury.

74. A method of claim 66, wherein said skeletal muscle disease is resulting from a neuropathy.

75. A method of claims 1, 3, 4, and 31, wherein said medicament is for treating a muscle cell in a patient with a cardiac muscle disorder.

76. A method of claim 75, wherein said cardiac disorder is cardiomyopathy.

77. A method of claim 75, wherein said cardiac disorder is ischemic damage.

78. A method of claim 75, wherein said cardiac disorder is a congenital disease.

79. A method of claim 75, wherein said cardiac disorder is cardiac trauma.

80. A method of claims 1, 3, 4, and 31, wherein said medicament is for treating a muscle cell in a patient with a smooth muscle disorder.

81. A method of claim 80, wherein said disorder is arterial sclerosis.

82. A method of claim 80, wherein said disorder is a vascular lesion.

83. A method of claim 80, wherein said disorder is a congenital vascular disease.

84. A method of claims 1, 3, 4, and 31, wherein said medicament is for treating a muscle cell which has insufficient functional acetylcholine receptors.

85. A method of claim 85 wherein said muscle cell lacking sufficient acetylcholine receptor is a muscle cell in a patient with myasthenia gravis.

86. A method as claimed in claim 84, wherein said condition involves muscular damage.

87. A method of making a medicament for the prophylaxis or treatment of a muscular tumor in a patient, said method comprising admixing an effective amount of a substance which inhibits the binding of a factor as defined in any one of claims 1, 3, 4, and 31 to a receptor therefor with a pharmaceutically acceptable carrier.

88. A method of making a medicament for treating a mammal suffering from a disease of muscle cell proliferation, said method comprising admixing an antibody which binds to a polypeptide of any of one of claims 1, 3, 4, and 31 with a pharmaceutically acceptable carrier.

89. A method of identifying a nucleic acid sequence coding for a molecule having muscle cell mitogenic activity, said method comprising contacting a cell containing sample with a muscle cell mitogen specific antibody to determine expression of said mitogen in said sample and isolating said nucleic acid sequence from the cells exhibiting said expression.

90. The method of claim 31, wherein said GGF2 is human recombinant GGF2.

91. A method of stimulating myogenesis of a muscle cell said method comprising contacting said muscle cell with a compound which specifically binds the $p185^{erbB2}$ receptor of muscle cells.

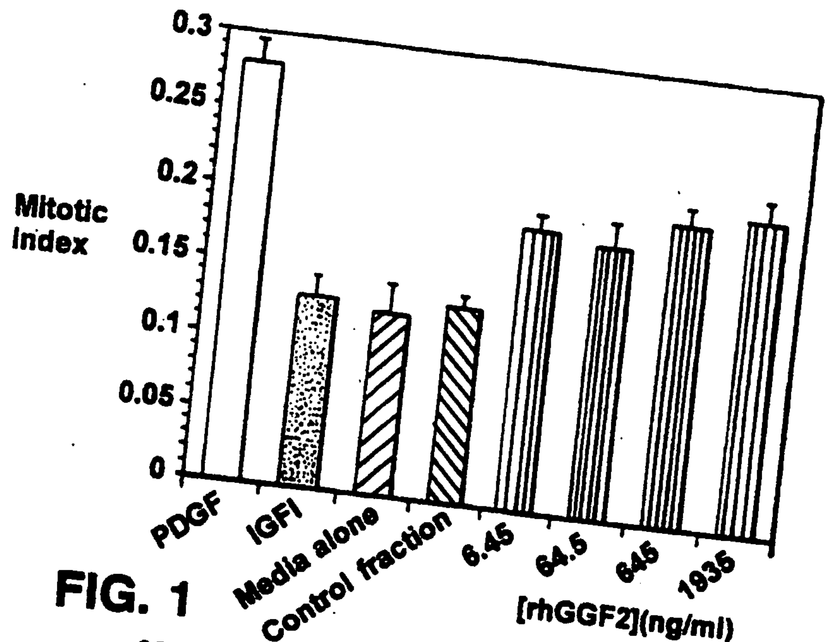

Mitotic
index
0.3
0.25
0.2
0.15
0.1
0.05
0
PDGF
IGFI
Media alone
Control fraction
6.45
64.5
645
1935
[rhGGF2](ng/ml)

FIG. 1

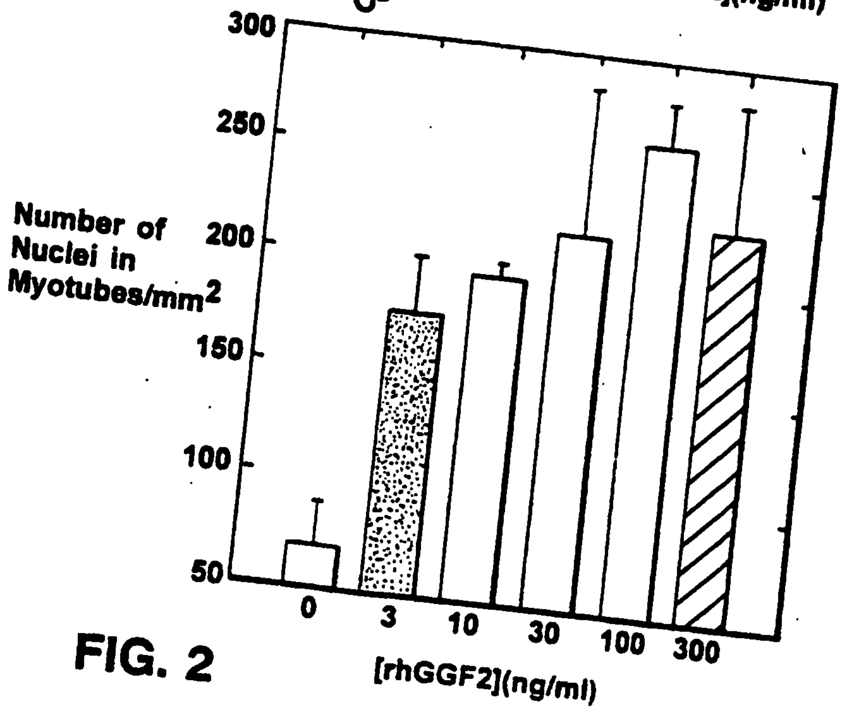

Number of
Nuclei in
Myotubes/mm2
300
250
200
150
100
50
0
3
10
30
100
300
[rhGGF2](ng/ml)

FIG. 2

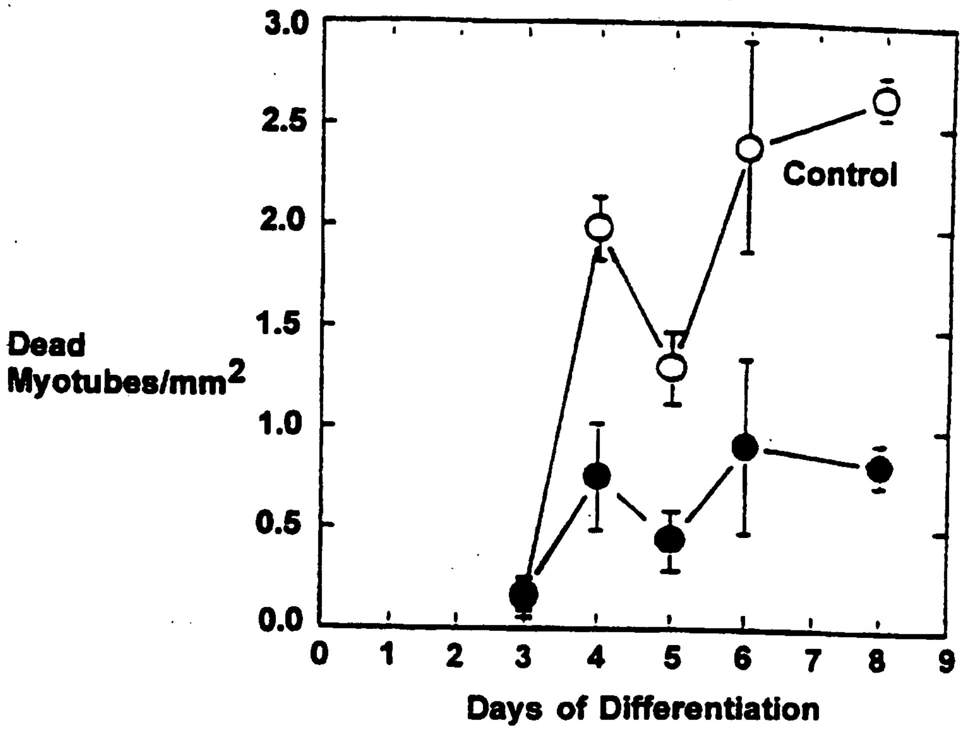

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Dead
Myotubes/mm2
Control
0 1 2 3 4 5 6 7 8 9
Days of Differentiation

FIG. 3

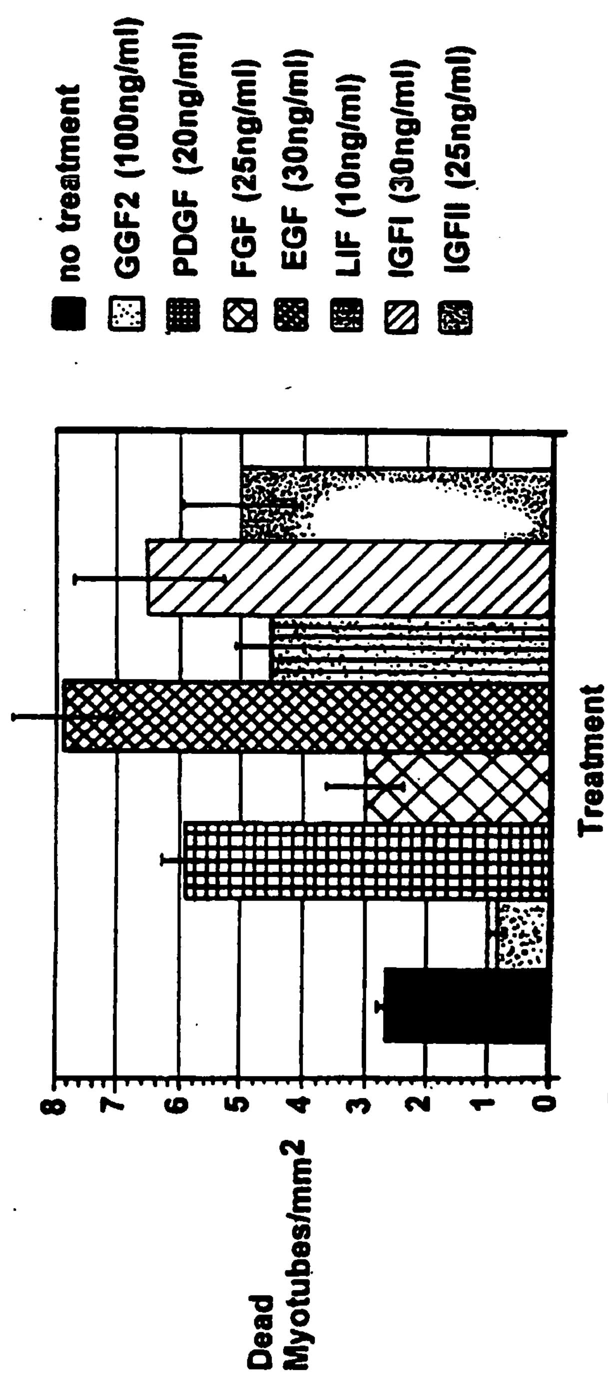

no treatment
GGF2 (100ng/ml)
PDGF (20ng/ml)
FGF (25ng/ml)
EGF (30ng/ml)
LIF (10ng/ml)
IGFI (30ng/ml)
IGFII (25ng/ml)
Dead Myotubes/mm2
0
1
2
3
4
5
6
7
8
Treatment

FIG. 4

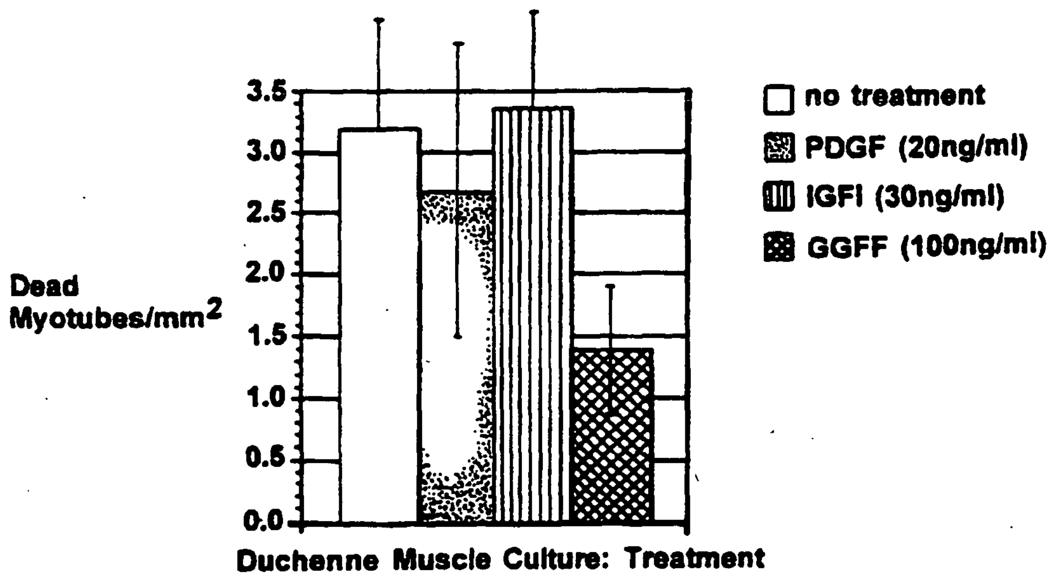

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Dead Myotubes/mm2
no treatment
PDGF (20ng/ml)
IGFI (30ng/ml)
GGFF (100ng/ml)
Duchenne Muscle Culture: Treatment

FIG. 5

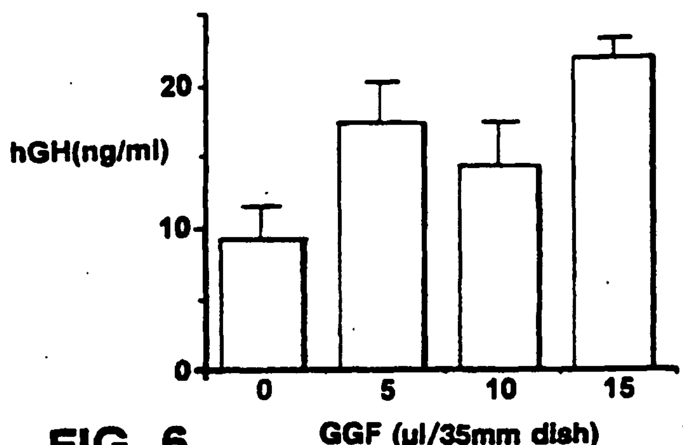

20
10
0
hGH(ng/ml)
0
5
10
15
GGF (µl/35mm dish)

FIG. 6

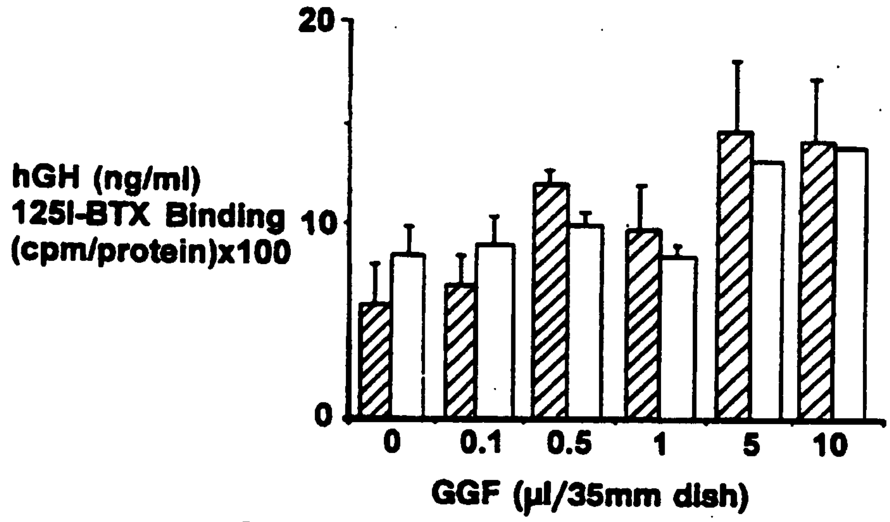

hGH (ng/ml)
125I-BTX Binding
(cpm/protein)x100
20
10
0
0
0.1
0.5
1
5
10
GGF (µl/35mm dish)

FIG. 7

FIG. 8

	N-terminus		
GGF-I 01	F K G D A H T E	(SEQ ID NO: 1)	
	Trypsin peptides		
GGF-I 02	K/R A S L A D E Y E Y M X K *	(SEQ ID NO: 2)	
GGF-I 03	K/R T E T S S S G L X L K *	(SEQ ID NO: 3)	
GGF-I 04	K/R K L G E M W A E	(SEQ ID NO: 4)	HMG-1
GGF-I 05	K/R L G E K R A	(SEQ ID NO: 5)	HMG-1?
GGF-I 06	K/R I K S E H A G L S I G D T A K *	(SEQ ID NO: 6)	HMG-2
GGF-I 07	K/R A S L A D E Y E Y M R K *	(SEQ ID NO: 7)	
GGF-I 08	K/R I K G E H P G L S I G D V A K *	(SEQ ID NO: 8)	HMG-1
GGF-I 09	K/R M S E Y A F F V Q T X R *	(SEQ ID NO: 9)	HMG-2
GGF-I 10	K/R S E H P G L S I G D T A K *	(SEQ ID NO: 10)	HMG-1
GGF-I 11	K/R A G Y F A E X A R *	(SEQ ID NO: 11)	
GGF-I 12	K/R K L E F L X A K *	(SEQ ID NO: 12)	
GGF-I 13	K/R T T E M A S E Q G A	(SEQ ID NO: 13)	
GGF-I 14	K/R A K E A L A A L K *	(SEQ ID NO: 14)	
GGF-I 15	K/R F V L Q A K K *	(SEQ ID NO: 15)	
GGF-I 16	K/R L G E M W	(SEQ ID NO: 16)	HMG-1
	Protease V8 peptides		
GGF-I 17	E T Q P D P G Q I L K K V P M V I G A Y T	(SEQ ID NO: 165)	
GGF-I 18	E Y K C L K F K W F K K A T V M	(SEQ ID NO: 17)	
GGF-I 19	E A K Y F S K X D A	(SEQ ID NO: 18)	LH alpha
GGF-I 20	E X K F Y V P	(SEQ ID NO: 19)	
GGF-I 21	E L S F A S V R L P G C P P G V D P M V S F P V A L	(SEQ ID NO: 20)	LH beta

FIG. 9

A

GGF-I 01	F K G D A H T E	(SEQ ID NO: 11)
GGF-I 02	A S L A D E Y E Y M X K	(SEQ ID NO: 22)
GGF-I 03	T E T S S S G L X L K	(SEQ ID NO: 23)
GGF-I 07	A S L A D E Y E Y M R K	(SEQ ID NO: 24)
GGF-I 11	A G Y F A E X A R	(SEQ ID NO: 25)
GGF-I 13	T T E M A S E Q G A	(SEQ ID NO: 26)
GGF-I 14	A K E A L A A L K	(SEQ ID NO: 27)
GGF-I 15	F V L Q A K K	(SEQ ID NO: 28)
GGF-I 17	E T Q P D P G Q I L K K V P M V I G A Y T	(SEQ ID NO: 29)
GGF-I 18	E Y K C L K F K W F K K A T V M	(SEQ ID NO: 17)

B

GGF-I 20	E X K F Y V P	(SEQ ID NO: 19)
GGF-I 12	K L E F L X A K	(SEQ ID NO: 12)

FIG. 10

	Trypsin peptides		
GGF-II 01	K/R V H Q V W A A K *		(SEQ ID NO: 42)
GGF-II 02	K/R Y I F F M E P E A X S S G		(SEQ ID NO: 43)
GGF-II 03	K/R L G A W G P P A F P V X Y		(SEQ ID NO: 44)
GGF-II 04	K/R W F V V I E G K *		(SEQ ID NO: 45)
GGF-II 05	K/R A L A A A G Y D V E K *	Histone H1	(SEQ ID NO: 164)
GGF-II 06	K/R L V L R *		(SEQ ID NO: 165)
GGF-II 07	K/R X X Y P G Q I T S N	Trypsin	(SEQ ID NO: 166)
GGF-II 08	K/R A S P V S V G S V Q E L V Q R *		(SEQ ID NO: 46)
GGF-II 09	K/R V C L L T V A A P P T		(SEQ ID NO: 46)
GGF-II 10	K/R D L L L X V		(SEQ ID NO: 50)
	Lysyl Endopeptidase-C peptides		
GGF-II 11	K V H Q V W A A K *		(SEQ ID NO: 48)
GGF-II 12	K A S L A D S G E Y M X K*		(SEQ ID NO: 49)

FIG. 11

A

GGF-II 01	V H Q V W A A K	(SEQ ID NO: 42)
GGF-II 02	Y I F F M E P E A X S S G	(SEQ ID NO: 43)
GGF-II 03	L G A W G P P A F P V X Y	(SEQ ID NO: 44)
GGF-II 04	W F V V I E G K	(SEQ ID NO: 45)
GGF-II 08	A S P V S V G S V Q E L V Q R	(SEQ ID NO: 46)
GGF-II 09	V C L L T V A A P P T	(SEQ ID NO: 47)
GGF-II 11	K V H Q V W A A K	(SEQ ID NO: 48)
GGF-II 12	K A S L A D S G E Y M X K	(SEQ ID NO: 49)

B Novel Factor II Peptides - others

GGF-II 10	D L L L X V	(SEQ ID NO: 50)

Comparisor. Br… JSA and [125 I]UdR …ntat …iethod for the DNA synthesis assay in Schwann cell cultures

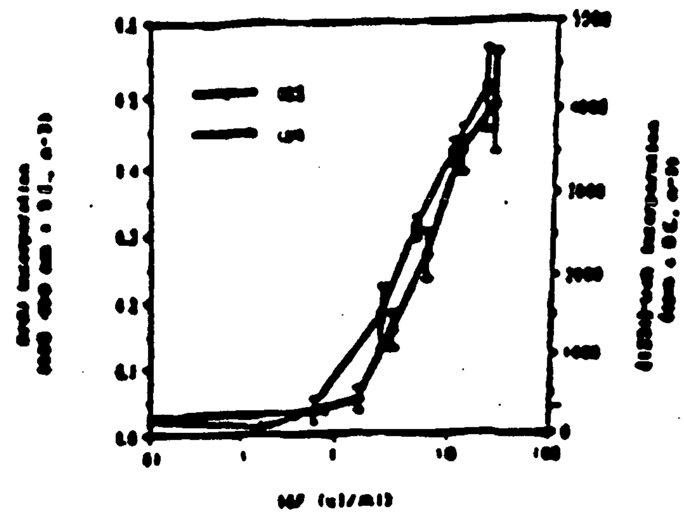


FIGURE 12

Comparison of Br-UdR immunoreactivity and Br-UdR labelled cell number

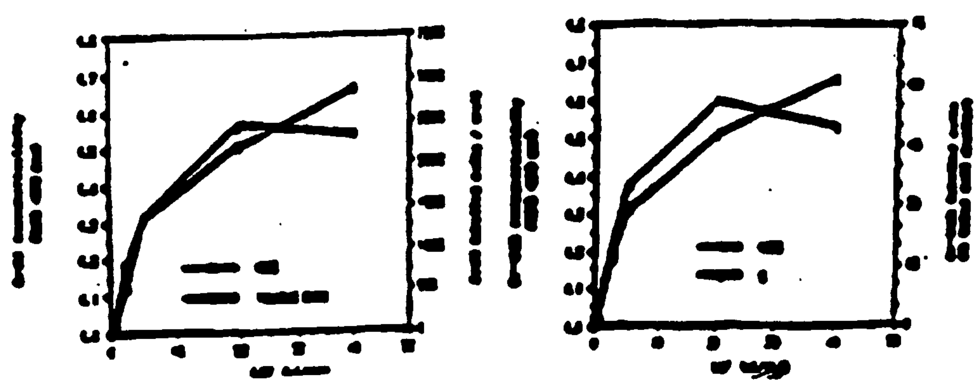


FIGURE 13A FIGURE 13B

BAD ORIGINAL

Mitogenic response of rat sciatic nerve Schwann cell toGGFs

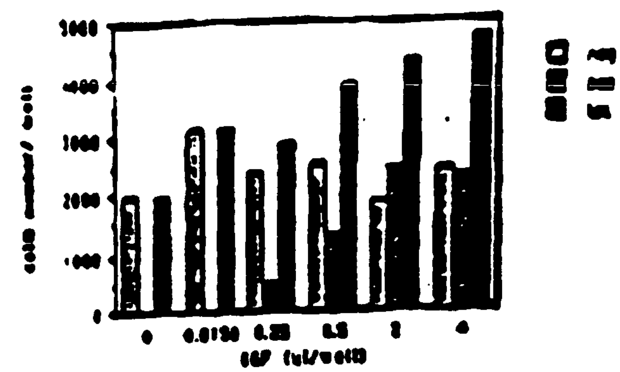


FIGURE 14

DNA synthesis in rat sciatic nerve Schwann cells and 3T3 fibroblasts in the presence of GGFs

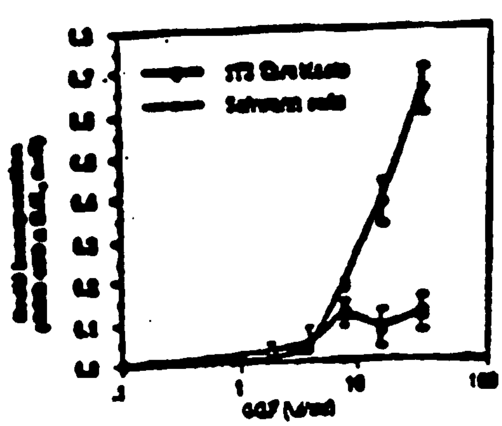


FIGURE 15

BAD ORIGINAL

Mitogenic response of BHK ᵤ C13 cells to FCS and GGFs

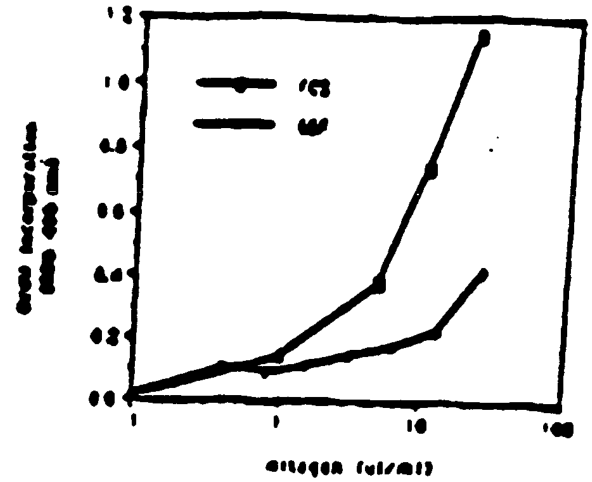


FIGURE 16

Survival and proliferation of BHK21 C13 cell microcultures after 48 hours in presence of GGFs

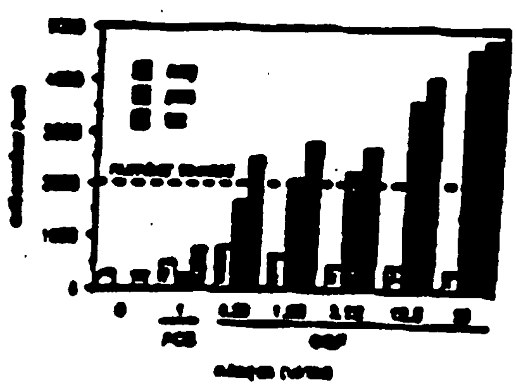


FIGURE 17

BAD ORIGINAL

Mitogenic response of C6 cells to FCS

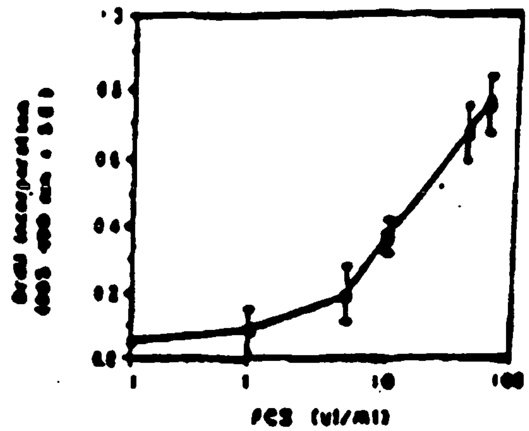


FIGURE 18

Mitogenic response of C6 cells to aFGF and GGFs

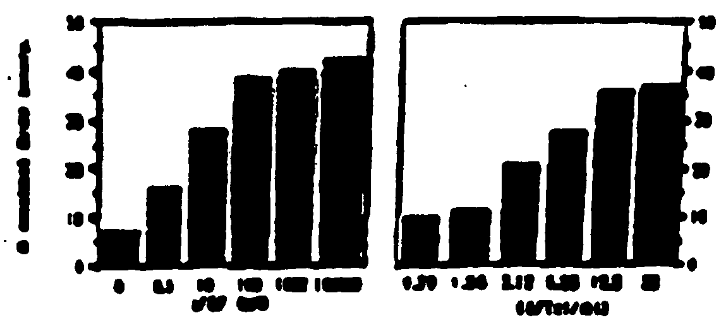


FIGURE 19

BAD ORIGINAL

DEGENERATE OLIGONUCLEOTIDE PROBES FOR FACTOR I AND FACTOR II

Oligo	Sequence	Peptide	
535	TTYAARGGNGAYCCNCAYAC!	GGFI-1	(SEQ ID NO: 51)
536	CATRTAYTCRTAYTCRTCNGC!	GGFI-2	(SEQ ID NO: 52)
537	TGYTCNGCNGCCATYTCNGT!	GGFI-13	(SEQ ID NO: 53)
538	TGYTCRCTNGCCATYTCNGT!	GGFI-13	(SEQ ID NO: 54)
539	CCDATNACCATNGGNACYTT!	GGFI-17	(SEQ ID NO: 55)
540	GCNGCCCARAACTTNGTNGAC!	GGFII-1	(SEQ ID NO: 56)
541	GCYTCNGGYTCCATRAARAA!	GGFII-3	(SEQ ID NO: 57)
542	CCYTCDATNACRACRAACCA!	GGFII-4	(SEQ ID NO: 58)
543	TCNGCRAARTANCCNGC!	GGFI-11	(SEQ ID NO: 59)
544	GCNGCNAGNGCYTCYTTNGC!	GGFI-14	(SEQ ID NO: 60)
545	GCNGCYAANGCYTCYTTNGC!	GGFI-14	(SEQ ID NO: 61)
546	TTYTTNGCYTGNAGNACRAA!	GGFI-15	(SEQ ID NO: 62)
551	TTYTTNGCYTGYAANACRAA!	GGFI-15	(SEQ ID NO: 63)
568	TGNACRAGYTCYTTNAC!	GGFII-6	(SEQ ID NO: 64)
569	TGNACYAAYTCYTTNAC!	GGFII-6	(SEQ ID NO: 65)
609	CATRTAYTCNCCNGARTCNGC!	GGFII-12	(SEQ ID NO: 66)
610	CATRTAYTCNCCRCTRTCNGC!	GGFII-12	(SEQ ID NO: 67)
649	NGARTCNGCYAANGANGCYTT!	GGFII-12	(SEQ ID NO: 68)
650	NGARTCNGCNAGNGANGCYTT!	GGFII-12	(SEQ ID NO: 69)
651	RCTRTCNGCYAANGANGCYTT!	GGFII-12	(SEQ ID NO: 70)
652	RCTRTCNGCNAGNGANGCYTT!	GGFII-12	(SEQ ID NO: 71)
653	NGARTCNGCYAARCTNGCYTT!	GGFII-12	(SEQ ID NO: 72)
654	NGARTCNGCNAGRCTNGCYTT!	GGFII-12	(SEQ ID NO: 73)
655	RCTRTCNGCYAARCTNGCYTT!	GGFII-12	(SEQ ID NO: 74)
656	RCTRCTNGCNAGRCTNGCYTT!	GGFII-12	(SEQ ID NO: 75)
659	ACNACNGARAAYGGNTCNGA!	GGFI-13	(SEQ ID NO: 76)
660	ACNACNGARAAYGGNAGYGA!	GGFI-13	(SEQ ID NO: 77)
661	CAYCARGTNTGGGCNGCNAA!	GGFII-1	(SEQ ID NO: 78)
662	TTYGTNGTNATHGARGGNAA!	GGFII-4	(SEQ ID NO: 79)
663	AARGGNGAYCCNCAYACNGA!	GGFI-1	(SEQ ID NO: 80)
664	GARGCNTTYGCNGCNTTNGA!	GGFI-14	(SEQ ID NO: 81)
665	GTNGCNTCNGTNCARAAYTT!	GGFII-8	(SEQ ID NO: 82)
666	GTNGCNAGYGTNCARAAYTT!	GGFII-8	(SEQ ID NO: 83)
694	NACYTTYTTNGARAAYTTNGC!	GGFI-17	(SEQ ID NO: 84)

FIGURE 20

BAD ORIGINAL

[illegible]

[illegible]

(SEQ ID NO: [illegible])

FIGURE 21

BAD ORIGINAL

FIGURE 22

PCR PRIMERS FOR FACTOR I AND FACTOR II

Degenerate PCR primers

Oligo	Sequence	Peptide	
657	CCGAATTCTGCAGGARAANCARCCNGAYCCNGG	GGFI-17	(SEQ ID NO: 86)
658	AAGGATCCTGCAGNGTRTANGCNCCNATNACCATNGG	GGFI-17	(SEQ ID NO: 87)
667	CCGAATTCTGCAGGCNGAYTCNGGNGARTAYATG	GGFII-12	(SEQ ID NO: 88)
668	CCGAATTCTGCAGGCNGAYATHGGNGARTAYAT	GGFII-12	(SEQ ID NO: 89)
669	AAGGATCCTGCAGNNNCATRTAYTCNCCNGARTC	GGFII-12	(SEQ ID NO: 90)
670	AAGGATCCTGCAGNNNCATRTAYTCNCCRATRTC	GGFII-12	(SEQ ID NO: 91)
671	CCGAATTCTGCAGGCAYCARGTNTGGGCNGCNAA	GGFII-1	(SEQ ID NO: 92)
672	CCGAATTCTGCAGATHTTYTTYATGGARCCNGARG	GGFII-8	(SEQ ID NO: 93)
673	CCGAATTCTGCAGGGNGGNCCNCCNGGNTTYCCNGT	GGFII-3	(SEQ ID NO: 94)
674	CCGAATTCTGCAGTGGTTYGTNGTNATHGARGG	GGFII-4	(SEQ ID NO: 95)
677	AAGGATCCTGCAGYTTNGCNGCCCANACYTGRTG	GGFII-1	(SEQ ID NO: 96)
678	AAGGATCCTGCAGGCYTCNGGYTCCATRAARAA	GGFII-8	(SEQ ID NO: 97)
679	AAGGATCCTGCAGAANGGRAANCCNGGNGGNCC	GGFII-3	(SEQ ID NO: 98)
680	AAGGATCCTGCAGYTTNCCYTCDATNACNACRAAC	GGFII-4	(SEQ ID NO: 99)
681	CATRTAYTCRTAYTCTGNGCAAGGATCCTGCAG	GGFI-8	(SEQ ID NO: 100)
682	CCGAATTCTGCAGAARGGNGAYGGNCAYACNGA	GGFI-1	(SEQ ID NO: 101)
683	GCNGCTAANGCYACYTTNGCAAGGATCCTGCAG	GGFI-14	(SEQ ID NO: 102)
684	GCNGCNAGNGCYTCYTTNGCAAGGATCCTGCAG	GGFI-14	(SEQ ID NO: 103)
685	TCNGCRAARTANCCNGCAAGGATCCTGCAG	GGFII-1	(SEQ ID NO: 104)

Unique PCR primers for Factor II

Oligo	Sequence	Comment	
711	CATCGATCTGCAGGCTGATTCTGGAGAATATATGTGCA	3' RACE	(SEQ ID NO: 105)
712	AAGGATCCTGCAGCCACAATCTCGAGTCGACATCGATT	3' RACE	(SEQ ID NO: 104)
713	CCGAATTCTGCAGTGATCAGCAAACTAGGAAATGACA	3' RACE	(SEQ ID NO: 105)
721	CATCGATCTGCAGCCTAGTTTGCTGATCACTTTGCAC	5' RACE	(SEQ ID NO: 106)
722	AAGGATCCTGCAGTATATTCTCCAGAATCAGCCAGTG	5' RACE; ANCHORED	(SEQ ID NO:
725	AAGGATCCTGCAGGCACGCAGTAGGCATCTCTTA	EXON A	(SEQ ID NO: 108)
726	CCGAATTCTGCAGCAGAACTTCGCATTAGCAAAGC	EXON A	(SEQ ID NO: 109)
771	CATCCCGGGATGAAGAGTCAGGAGTCTGTGGCA	EXONS B+A	(SEQ ID NO: 110)
772	ATACCCGGGCTGCAGACAATGAGATTTCACACACCTGCG		(SEQ ID NO: 111)
773	AAGGATCCTGCAGTTTGGAACCTCCCACAGACTCCT	ANCHORED	(SEQ ID NO: 112)
776	ATACCCGGGCTGCAGATGAGATTTCACACACCTGCGTGCA	EXONS B+A	(SEQ ID NO: 113)

BAD ORIGINAL

BAD ORIGINAL

Summ ·· of contiguous GGF-II cDNA struct ·· s and sequences

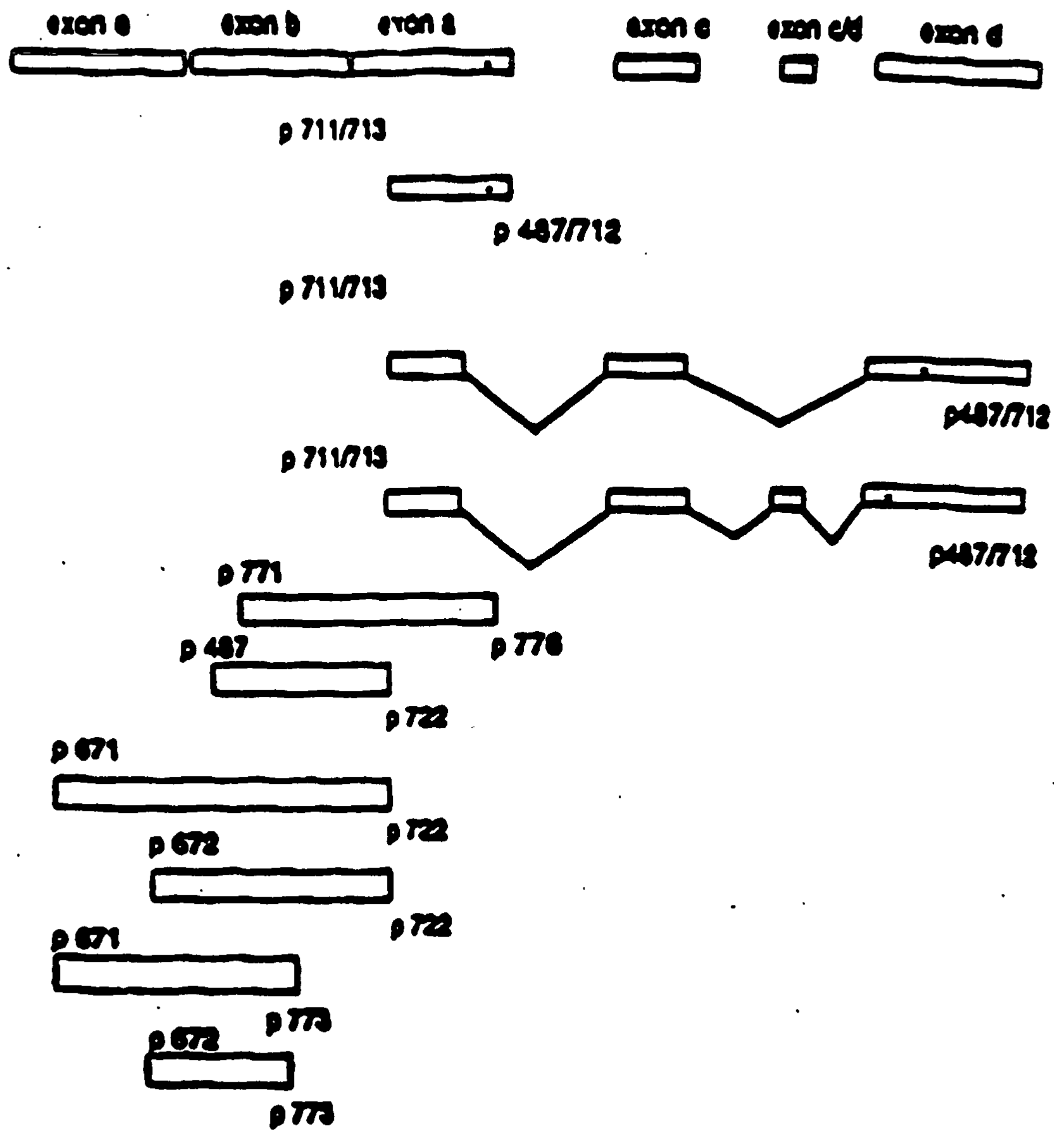
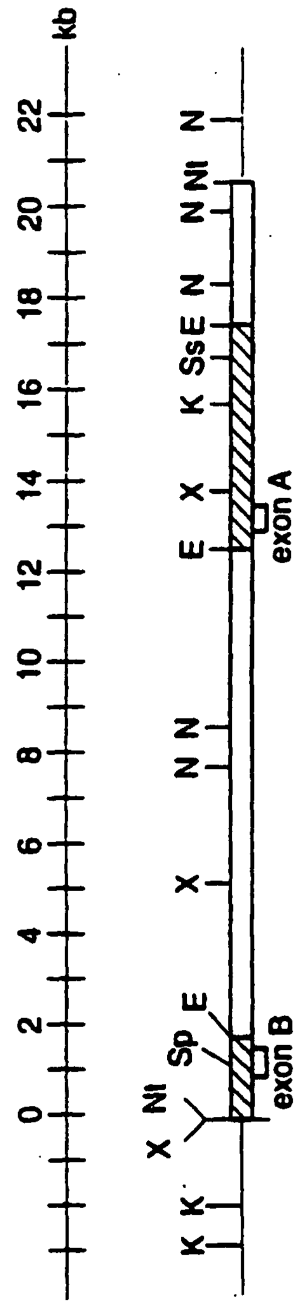


FIGURE 23

BAD ORIGINAL

FIG. 24


kb
0
2
4
6
8
10
12
14
16
18
20
22
K K
X Nl
Sp
E
X
N N
E X
K Ss E
N
N Nl
N
exon A
exon B

Alternative gene products of putative bovine GGF-II

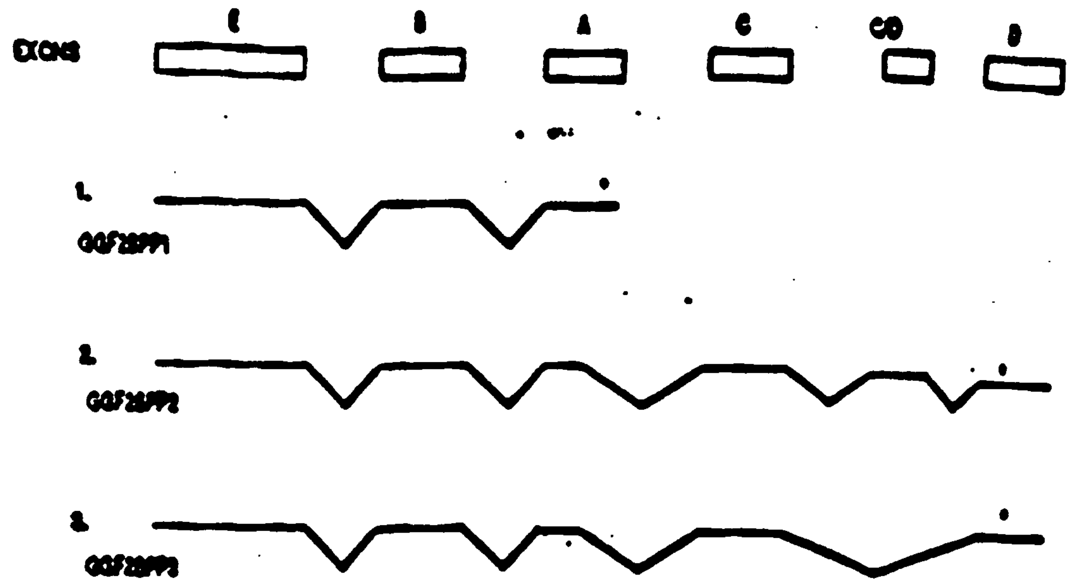


FIGURE 25

BAD ORIGINAL

OGP-II pe[illegible] [illegible]ntified in deduce[illegible] [illegible] [illegible]id sequences of putativ bo[illegible]ne OGP-II proteins

Peptide	Pos.	Sequence match	
II-1		VHQVVAAK	
	1:	HQVVAAK AAGLK	(SEQ ID NO: 116)
II-10		DLLLKV	
	14:	CGLKK dellkv RLGAN	(SEQ ID NO: 117)
II-03		LGAVGPPAFPVKY	(SEQ ID NO: 118)
	21:	LLTVR lgavghpafpeog RLKED	(SEQ ID NO: 119)
II-02		YIPFMEPEAXSSG	(SEQ ID NO: 120)
	41:	KEDSR YIPTMEPEANSSG CPGRL	(SEQ ID NO: 121)
II-6		LVLR	
	103:	VAGSK LVLR CETSS	(SEQ ID NO: 122)
I-18		EYKCLKFKVFKQATVK	(SEQ ID NO: 123)
	112:	CETSS eysslkfkvfkngsel SRNMK	(SEQ ID NO: 124)
II-12		KASLADSGEYNCK	(SEQ ID NO: 125)
	151:	ELRIS KASLADSGEYNCK VISKL	(SEQ ID NO: 126)
I-07		ASLADEYEYNGK	(SEQ ID NO: 127)
	152:	LRISK asladsqeymck VISKL	(SEQ ID NO: 128)

FIGURE 26

BAD ORIGINAL

[illegible]

(SEQ ID NO: 129)

FIGURE 27 1/3

BAD ORIGINAL

[illegible]

[illegible]

(SEQ ID NO: 130)

FIGURE 27 2/3

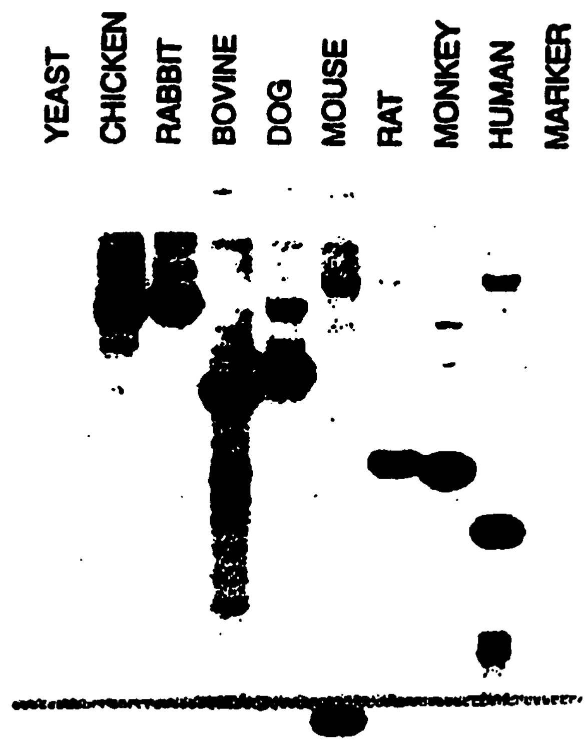
BAD ORIGINAL

[illegible]

(SEQ ID NO: 13)

FIGURE 27 3/3

FIG. 28


YEAST
CHICKEN
RABBIT
BOVINE
DOG
MOUSE
RAT
MONKEY
HUMAN
MARKER

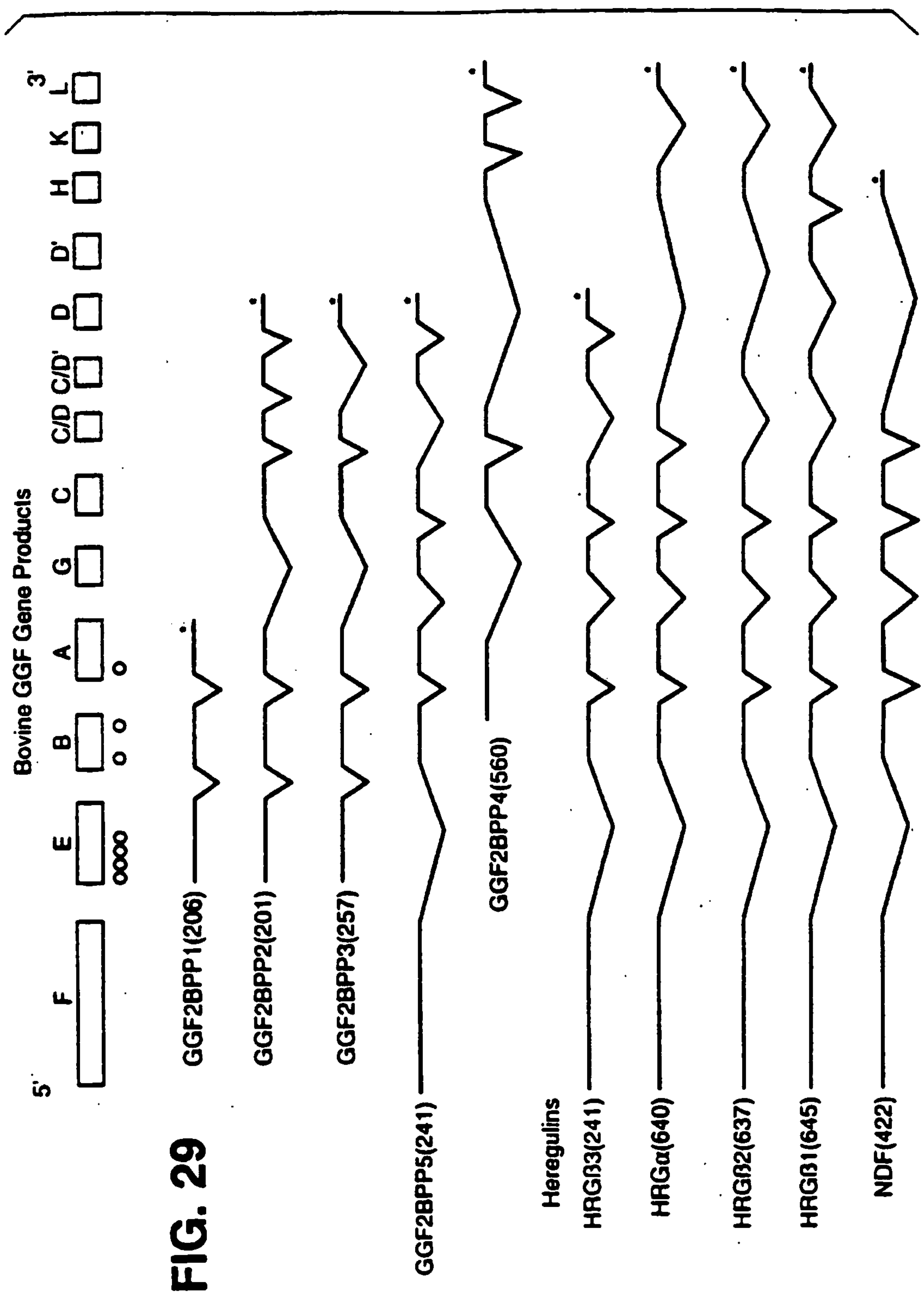

Bovine GGF Gene Products
5'
F
E
B
A
G
C
C/D
C/D'
D
D'
H
K
L
3'
GGF2BPP1(206)
GGF2BPP2(201)
GGF2BPP3(257)
GGF2BPP5(241)
GGF2BPP4(560)
Heregulins
HRGβ3(241)
HRGα(640)
HRGβ2(637)
HRGβ1(645)
NDF(422)

FIG. 29

FIGURE 30 1/

CODING SEGMENTS OF GLIAL GROWTH FACTOR/HEREGULIN GENE

CODING SEGMENT F: (SEQ ID NO: 132)

```
AGTTTCCCCCCCCAACTTGTCGGAACTCTGGGCTCGCGCGCAGGGCAGGAGCGGAGCGGC   60

GGCGGCTGCCCAGGCGATGCGAGCGCGGGGCCGGACGGTAATCGGCCTCTCCCGTCCTCGGGG  120

TGCGAGCGCGCCCGGACCGAGGCAGCGACAGGAGCGGACCGCGGCGGGAACCGAGGACTCC  180

CCAGCGGCGCCCCAGCAGGAGCCACCCCGCGAGnCGTGCGACCCGGGACCGCAGCGCCCCGCC  240

AGTCCCAGGTCGCCCCGGACCGCACGTTGCGTCCCCGCGCTCCCCGCCGGCCGACAGGAGAC  300

GCTCCCCCCCCACGCCGCGCGCGCCTCGGCCCGGTCGCTGGCCCGGCCTCCACTCCGGGGAC  360
             |||| ||||||| || ||| |||| || | |  || |||  |||||
             cgcgagcgcctcagcgcggccgctcgctctc..cccctcgagggac

AAACTTTTCCCGAAGCCGATCCCAGCCCTCGGACCCAAACTTGTCGCGCGTCGCCTTCGC  420
|||||||||| || ||||||| |||||| ||||| ||           |||||| |||
aaacttttcccaaacccgatccgagcccttggaccaaa...........ctcgcctgcgc

                                       M  S  E  R  K  E  G
CGGGAGCCGTCCGCGCAGAGCGTGCACTTCTCGGGCGAGATGTCGGAGCGCAAAGAAGGC  480
|| |||||||||||| ||||||   | | |||| |||||||||||| ||||||| |||||||
cgagagccgtccgcgtagagcgctc.cgtctccggcgagatgtccgagcgcaaagaaggc
                                                      K

K  G  K  G  K  G  G  K  K  D  R  G  S  G  K  K  P  V  P  A
AAACGGCAAGGGGAAGGGCGGCAAGAAGGACCGAGGCTCCGGGAAGAAGCCCGTGCCCGCG  540
| |||||| ||||||||    |||||||| |||||||||||| |||||||| | |||||
agaggcaaagggaagggcaagaagaaggagcgaggctccggcaagaagccggagtccgcg
R                    K        E                         E  S

A  G  G  P  S  P  A
GCTGGCGGCCCCAGCCCAG   559
|| ||| ||| ||||||||
gcggggcagccagagcccag
```

CODING SEGMENT E: (SEQ ID NO: 133)

```
CCCATCAAGTGTCGGCGGCGAAAGCCGCGGGGCTTGAAGAAGGACTCGCTGCTCACCGTGC   60
 M  Q  V  W  A  A  K  A  G  G  L  K  K  D  S  L  L  T  V  R

GCCTGGGCGCCTGGGGCCACCCCGCCTTCCCCTCCTGCGGGCGCCTCAAGGACGACAGCA  120
 L  G  A  W  G  H  P  A  F  P  S  C  G  R  L  K  E  D  S  R

GGTACATCTTCTTCATGGAGCCCGAGGCCAACAGCAGCGGCGCGCCCGGCCGCCTTCCGGA  180
 Y  I  F  F  M  E  P  E  A  N  S  S  G  G  P  G  R  L  P  S

GCCTCCCTTCCCCCCTCTCCGACGACGGCCCCGAACCTCAAGAAGCAGGTCAGCCCGGTGCTG  240
 L  L  P  P  S  R  D  G  P  E  P  Q  E  G  G  Q  P  G  A  V

TCCAACG[illegible]   [illegible]
```

BAD ORIGINAL

FIGURE 30 2/8

CODING SEGMENT B: (SEQ ID NO: 134)

```
 L  P  P  R  L  K  E  N  K  S  Q  E  S  V  A  G  S  K  L  V
CCTTGCCTCCCCCGCTTGAAAGAGATGAAGAGTCAGGAGTCTGTGGCAGGTTCCAAACTAG   60
(alignment bars)
ccttgcctccccgattgaaagagatgaaaagccaggaatcggctgcaggttccaaactag
      Q                                A

 L  R  C  E  T  S  S  E  Y  S  S  L  K  F  K  W  F  E  N  G
TGCTTCGGTGCCGAGACCAGTTCTGAATACTCCTCTCTCAAGTTCAAGTGGTTCAAGAATG  120
(alignment bars)
tccttcggtgtgaaaccagttctgaatactcctctctcagattcaagtggttcaagaatg
                                        R

 S  E  L  S  R  K  N  K  P  Q  N  I  K  I  Q  K  R  P  G
GGAGTGAATTAAGCCGAAAGAACAAACCACAAAACATCAAGATACAGAAAAGGCCCGGG   178
(alignment bars)
ggaatgaattgaatcgaaaaaacaaaccacaaaatatcaagatacaaaaaagccagg
 N     N                                        K
```

CODING SEGMENT A: (SEQ ID NO: 135)

```
 K  S  E  L  R  I  S  K  A  S  L  A  D  S  G  E  Y  N  C  E
GAAGTCAGAACTTCGGCATTAGCAAAGCGTCACTGGCTGATTCTGGAGAATATAATGTGCAA   60
(alignment bars)
gaagtcaggagcttcgcattaacaaagcatcactggctgattctggagagtatatgtgcaa
                   N

 V  I  S  K  L  G  N  D  S  A  S  A  N  I  -  I  V  E  S  N
AGTGATCAGCAAACTAGGAAATGACAGTGCCTCTGCCAACATCACCATTGTGGAGTCAAA   120
(alignment bars)
agtgatcagcaaattaggaaatgacagtgcctctgccaatatcaccatcgtggaatcaaa

 A
CG   122
||
cg
```

CODING SEGMENT A'1 (SEQ ID NO: 136)

```
TCTAAAACTACAGAGACTGTATTTTCATGATCATCATAGTTCTGTGAAATATATACTTAAAC   60
                            E  S  E  L  R  I  S  K  A  S  L  A  D
CGCTTTGCTCCTGATCTTGTAGGAAGTCAGAACTTCGGCATTAGCAAAGCGTCACTGGCTG   120
 S  G  E  Y  N  C  K  V  I  S  K  L  G  N  D  S  A  S  A  N
ATTCTGGAGAATATATATGTGCAAAGTGATCAGCAAACTAGGAAATGACAGTGCCTCTGCCA  180
 I  T  I  V  E  S  N  G  K  R  C  L  L  R  A  I  S  Q  S  L
ACATCACCATTGTGGAGTCAAACGGTAAGACATGCCCTACTGCCGTGCTATTTCTCAGTCTC  240
 R  G  [illegible]
[illegible]
```

BAD ORIGINAL

FIGU E 30 3/8

TGTGAACAAATAAAAATCATGAAACGAAAACTCTATGTTTGAAATATCTTATGCGTCCTC 360
CTGTAAAGCTCTTCACTCCATAAGGTGAAATAGACCTGAAATATATATATAGATTATTT 417

CODING SEGMENT G1: (SEQ ID NO: 137)

```
R  I  T  T  G  M  P  A  S  T  E  T  A  Y  V  S  S  E  S  P  I
AGATCACCACTGGCATGCCAGCCTCAACTGAGACAGCGTATGTGTCTTCAGAGTCTCCCA   60
|||||| |||||| ||||||||||||||||||     ||| |||||||||||||||||||||
agatcatcactggtatgccagcctcaactgaaggagcatatgtgtcttcagagtctccca
     I                                G

 R  I  S  V  S  T  E  G  T  N  T  S  S  S
TTAGAATATCAGTATCAACAGAAGGAACAAATACTTCTTCAT   102
|||||||||||||||| ||||||||| ||||||||||||||||
ttagaatatcagtatccacagaaggagcaaatacttcttcat
                          A
```

CODING SEGMENT C1: (SEQ ID NO: 156)

```
 T  S  T  S  T  A  G  T  S  H  L  V  K  C  A  E  K  E  K  I
CCACATCCACATCTACAGCTGGGACAAGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAA   60
| ||||| ||||| ||  |||||||||||||||||||| || ||||| ||||||||||||||
ctacatctacatccaccactgggacaagccatcttgtaaaatgtgcggagaaggagaaa
                 T

 F  C  V  N  G  G  E  C  F  M  V  K  D  L  S  N  P  S  R  Y
CTTTCTGTGTCAATGGAGGCGAGTGCTTCATGGTGAAAGACCTTTCAAATCCCTCAAGAT   120
||||||||||||||||||| |||||||||||||||||||||||||||||||| ||||| ||||
ctttctgtgtgaatggagggggagtgcttcatggtgaaagacctttcaaacccctcgagat

 L  C
ACTTGTGC   128
||||||||
acttgtgc
```

BAD ORIGINAL

FIGURE 30 4/8

CODING SEGMENT C/D: (SEQ ID NO: 138)

```
K  C  Q  P  G  F  T  G  A  R  C  T  E  N  V  P  M  K  V  Q
AAGTGCCAACCTGGATTCACTGGAGCGAGATGTACTGAGAATGTGCCCATGAAAGTCCAA  60
|||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
aagtgccaacctggattcactggagcaagatgtactgagaatgtgcccatgaaagtccaa

T  Q  E
ACCCAAGAA  69
| |||||||
aaccaagaa
N
```

CODING SEGMENT C/D': (SEQ ID NO: 139)

```
K  C  P  N  E  F  T  G  D  R  C  Q  N  Y  V  N  A  S  F  Y
AAGTGCCCAAATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGCCAGCTTCTAC  60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
aagtgcccaaatgagtttactggtgatcgctgccaaaactacgtaatgccagcttctac
```

CODING SEGMENT D: (SEQ ID NO: 140)

```
S  T  S  T  P  F  L  S  L  P  E  *
AGTACGTCCACTCCCTTTCTGTCTCTGCCTGAATAG  36
||||||||||||||||||||||||||||||||||||
agtacgtccactccctttctgtctctgcctgaatag
```

CODING SEGMENT D': (SEQ ID NO: 141)

```
K  H  L  G  I  E  F  M  V
aagcatcttgggattgaatttatggtg  27
```

BAD ORIGINAL

FIGURE 30 5/8

CODING SEGMENT 8: (SEQ ID NO: 142)

```
K  A  R  S  L  Y  Q  K  R  V  L  T  I  T  G  I  C  I  A  L
AAAGCCGCAGGAGCTCTACCAGAAGACAGTGCTCACCATTACCGGCATTTGCATCGCGCTG   60
|| |||||||||| |||||||||||||||| ||||| |||||||| |||||||| ||
aaggcggaggagctgtaccagaagagagtgctgaccataaccggcatctgcatcgccctg

L  V  V  G  I  H  C  V  V  V  Y  C  K  T  K  K  Q  N  K  K
CTCGTGGTTGGCATCATGTGTGTGGTGGTCTACTGCAAAACCAAGAAACAACGGAAAAAG   120
|| ||||| |||||||||||||||||||| ||||||||||||||||||||||| ||||||||
cttgtggtcggcatcatgtgtgtggtggcctactgcaaaaccaagaaacagcggaaaaag
                            A

L  H  D  R  L  R  Q  S  L  R  S  E  R  N  T  M  M  N  V  A
CTTCATGACCGGCTTCGGCAGAGCCTTCGGTCTGAAAGAAACACCATGATGAACGTAGCC   180
|| |||||||| |||||||||||||||||||||||||| |||||| ||||||||| | |||
ctgcatgaccgtcttcggcagagccttcggtctgaacgaaacaatatgatgaacattgcc
                                           N           I

N  G  P  H  H  P  N  P  P  P  E  N  V  Q  L  V  N  Q  Y  V
AACGGGCCCCACCACCCCAATCCGCCCCCCGAGAACGTGCAGCTGGTGAATCAATACGTA   240
|| ||||| ||||| || || || ||||||||||| || ||||||||||||||||||||||
aatgggcctcaccatcctaacccactcccccgagaatgtccagctggtgaatcaatacgta

S  K  N  V  I  S  S  E  H  I  V  E  R  E  A  E  S  S  P  S
TCTAAAAATGTCATCTCTAGCGAGCATATTGTTGAGAGAGAGGCCGAGAGCTCTTTTTCC   300
|||||||| |||||||| || ||||||||||||||||||||||| || ||||  || ||||||
tctaaaaacgtcatctccagtgagcatattgttgagagagaagcagagacatccttttcc
                                                  T

T  S  H  Y  T  S  T  A  S  H  S  T  T  V  T  Q  T  P  S  H
ACCAGTCACTACACTTCGACAGCCTCATCATTCCACTACTGTCACTCAGACTCCCAGTCAC   360
||||||||||| ||||| ||||| ||||| ||||||||||||||| |||||||| || |||
accagtcactatacttccacagccccatcactccactactgtcacccagactcctagccac

S  V  S  N  G  H  T  E  S  I  I  S  E  S  E  S  V  I  V  M
AGCTCGAGCAATGGACACACACTGAAAGCATCATTTCGGAAAGCCACTCTGTCATCGTGATG   420
|||||||||| |||||||||||||||||||| |||| ||||||||||||||| |||||||||
agctggagcaacggacacactgaaagcatcctttccgaaagccactctgtaatcgtgatg
                            L

S  S  V  E  N  S  R  H  S  S  P  T  G  G  P  R  G  R  L  N
TCATCCGTAGAAAACAGTAGGCACAGCAGCCCCGACTGGGGGCCCCAGAGGACGTCTCAAT   480
|||||||||||||||||||||||||||||||||| ||||||||||| ||||||||||| |||
tcatccgtagaaaacagtaggcacagcagccccaactgggggcccaagaggacgtcttaat

G  L  G  G  P  R  E  C  N  S  F  L  R  H  A  R  E  T  P  D
GGCTTGGGAGGCCCTCGTGAATGTAACAGCTTCCTCAGGCATGCCAGAGAAACCCCTGAC   540
|||    ||||||||||||||||||||||||||||||||||||||||||||||||||||||
ggcacaggaggccctcgtgaatgtaacagcttcctcaggcatgccagagaaacccctgat
  T

S  Y  R  D  S  P  H  S  E  R
TCCTACCGAGACTCTCCTCATAGTGAAAG   569
|||||||||||||  ||||||||||||||
[illegible]
```

BAD ORIGINAL

CODING SEGMENT (SEQ ID NO: 157) FIGURE 30 6/8

```
ACATAACCTTATAGCTCAGCTAACCACAAACAACGCCCACAGATCCAAAtgCATGCACAT
 N  N  L  I  A  S  L  R  S  N  K  A  N  R  S  S  C  H  Q  I
CCAgcTTTCCGCAACTCATCTTAGACCTTCTTCCATTCCCCATTGGCCTTCATTCTCTAA
 Q  L  S  A  T  N  L  R  A  S  S  I  P  H  W  A  S  F  S  N
GACCCCTTGGCCTTtAgCAAG
 T  P  W  P  L  G  R
```

CODING SEGMENT L1 (SEQ ID NO: 143)

```
 Y  V  S  A  M  T  T  P  A  R  M  S  P  V  D  F  H  T  P  S
GTATGTATCAGCAATGACCACCCCCGCTCGTATGTCACCTGTAGATTTCCACACGCCCAG   60
|||||| ||||| |||||||||||||||||||||||||||||||||||||||||||||||
gtatgtgtcagccatgaccaccccggctcgtatgtcacctgtagatttccacacgcccag

 S  P  K  S  P  P  S  E  N  S  P  P  V  S  S  T  T  V  S  N
CTCCCCCAAGTCACCCCCGTTCGGAAATGTCCCCGCCCCGTGTCCAGCACGACGGTCTCCAG  120
||||||||| || |||||||||||||||| || ||||||||||||| |||||| |||||
ctccccaaatcgcccccttcggaatgtctccacccgtgtccagcatgacggtgtccat
                                               N

 P  S  M  A  V  S  P  F  V  E  E  E  R  P  L  L  L  V  T  P
GCCCTCCATGGCGGTCAGTCCCTTCGTGGAAGAGGAGAGACCCCTGCTCCTTGTGACGCC   180
||| |||||||||||||| |||||| ||||||| |||||||| || || || ||||| ||
gccttccatggcggtcagccccttcatggaagaagagagacctctacttctcgtgacacc
                         M

 P  R  L  R  E  E  -  Y  D  H  H  A  Q  Q  F  H  S  F  H  C
ACCACGGCTGCGGGAGAAG...TATGACCACCACGCCCAGCAATTCAACTCGTTCCACTG   240
|||| ||||||||||||||    | |||| | ||| | |||||| || | ||| ||||||
accaaggctgcgggagaagaagtttgaccatcaccctcagcagttcagctccttccacca
                    K  F           P           S           H

 N  P  A  H  E  S  I  S  L  P  P  S  P  L  E  D  V  E  D  E
CAACCCCGCGCATGAGAGCAACAGCCTGCCCCCCAGCCCCCTTGAGGATAGTGGAGGATGA  300
||||||||||||||| || |||||||| || | ||||||||||||||||||||||||||
caaccccgcgcatgacagtaacagcctccctgctagccccttgaggatagtggaggatga
               D              A

 E  Y  E  T  T  Q  E  Y  E  P  A  Q  E  P  V  K  K  L  T  H
GGAATATGAAACGACCCAGGAGTACGAACCAGCTCAGGAGCCCGTTAAGAAACTCACCCA   360
||| ||||||||||||||| |||||||| ||||| |||||||| |||||||||||| ||||
ggagtatgaaacgacccaagagtacgagccagcccaagagcctgttaagaaactcgccca
                                                       A

 S  S  R  R  A  K  R  T  K  P  N  G  H  I  A  N  R  L  E  N
CAGCAGCCCGGCGGGCCAAAAGAACCAAGCCCAATGGTCACATTGCCCACAGGTTGGAAAT  420
   ||||||||||||||||||||||||||||:|||||| |!:|||||| |||| |||||: :
...tagccggcgggccaaaagaaccaagcccaatggccacattgctaacagattggaagt
                                                 N        V
```

BAD ORIGINAL

FIC 'RE 30 7/8

```
 D  N  N  T  G  A  D  S  S  N  S  S  S  E  T  E  D  E  R  V
CGACAACAACACAGGCGCTGACAGCAGTAACTCAGACAGCGAAACAGACGATGAAAGAGT   480
||||| ||||||| || |   | ||||||||||||||||| |||||||| ||||||||||
ggacagcaacacaagctcccagagcagtaactcagagagtgaaacagaagatgaaagagt
    S          S  S  Q

 G  E  D  T  P  P  L  A  I  Q  N  P  L  A  A  S  L  E  A  A
AGGAGAAGATACGCCTTTCCCTGGCCATACAGAACCCCCTGGCAGCCAGTCTCGAGGCCGC   540
||| |||||||||||||||||| ||||||||||||||||||||||||||| ||||| |
aggtgaagatacgcctttcctgggcatacagaacccccctggcagccagtcttgaggcaac
                      G                                    T

 P  A  P  R  L  V  D  S  R  T  N  P  T  G  G  P  S  P  Q  E
CCCTGCCTTCCCGCCTGGTCGACAGCAGGACTAACCCAACAGGCGGCCTTCTCTCCCGCAGGA   600
 ||||||||||||||| |||||||||||||||||| ||||| ||||||| | |||||
acctgccttcccgcctggctgacagcaggactaacccagcaggccgcttctcgacacagga
                 A                        A     R         T

 E  L  Q  A  R  L  S  G  V  I  A  N  Q  D  P  I  A  V  *
AGAATTGCAGGCCAGGCTCTCCGGTGTAATCGCTAACCAAGACCCTATCGCTGTCTAAAA   660
|||| | ||||||||||| ||  ||||||| |||||||||||||||||| ||||| |||||
agaaatccaggccaggctgtctagtgtaattgctaaccaagaccctattgctgtataaaa
    I             S

CCGAAATACACCCATAGATTCACCTGTAAAACTTTATTTTATATAATAAAGTATTCCAGC   720
|| ||||| || ||||||||||||||||||||||||||||||||||||||||||||||||
cctaaataaacacatagattcacctgtaaaactttattttatataataaagtattccagc

TTAAATTAAACAA   733
|||||||||||||
ttaaattaaacaa
```

BAD ORIGINAL

FIGURE 30 8/8

Human Coding Segment E: (SEQ ID NO: 159)

[illegible] 60
M R W R R A P R R S G R P G P R A Q R P

[illegible] 120
G S A A R S S P P L P L L P L L L L G

[illegible] 180
T A A L A P G A A A G N E A A P A G A S

[illegible] 240
V C Y S S P P S V G S V Q E L A Q R A A

[illegible] 300
V V I E G K V E P Q R R Q Q G A L D R K

[illegible] 360
A A A A A G E A G A W G G D R S P P A A

[illegible] 420
G P R A L G P P A E E P L L A A N G T V

[illegible] 480
P S W P T A P V P S A G E P G E E A P Y

[illegible] 540
L V K V E Q V W A V E A G G L E E D S L

[illegible] 600
L T V R L G T W G S P A F P S C G R L K

[illegible] 660
E D S R Y I F F M E P D A N S T S R A P

[illegible] 720
A A F R A S F P P L E T G R N L K K E V

[illegible] 749
S R V L C E R C

(SEQ ID NO: 147)

FIGURE 31 1/2

GGF2BPP5 nucleotide sequence and deduced protein sequence

```
AGTTTCCCCCCCCCAACTTGTCGGAACTCTGGGCTCGCGCGCAGGGCAGGAGCGGAGCGGC   60
GGCGGCTGCCCAGGCGATGCCAGCGCGCGGCCGGACGGTAATCGGCCTCTCCCTCCTCGGGC   120
TGCGAGCGCGCCCGGACCCGAGGCAGCGACAGGAGCGGACCGCGGCGGGAACCCAGGACTCC   180
GCAGCGGCGGCCCAGGCAGGAGCCACCCCGGCGAGNCGTGCGAACCCGGACGGAGCCCCCCGC   240
AGTCCCAGGTCGGCCCCGGACCCGCACGTTGCGTCCCCGCGCTCCCCGCCCGGCGACAGGAGAC   300
GCTCCCCCCCACGCCCCGCGCGCCTCGGCCCGGTCGCTGGCCCGCCTCCACTCCGGGGAC   360
AAACTTTTCCCGAAGCCGATCCCAGCCCTCGGACCCAAACTTGTCGCGCGTCGCCTTCGC   420
CGGGAGCCGTCCGCGCAGAGCGTGCACTTCTCGGGCGAGATGTCGGAGCGCAGAGAAGGC   480
                                    M  S  E  R  K  E  G
AAAGGCAAGGGGAAGGGCGGCAAGAAGGACCGAGGCTCCGGGAAGAAGCCCGTGCCCGCG   540
K  G  K  G  K  G  G  K  K  D  R  G  S  G  K  K  P  V  P  A
GCTGGCGGCCCGAGCCCAGCCTTGCCTCCCCGGCTTGAAAGAGATGAAGAGTCAGGAGTCT   600
A  G  G  P  S  P  A  L  P  P  R  L  K  E  M  K  S  Q  E  S
GTGGCAGGTTCCAAACTAGTGCTTCGGTGCGAGACCAGTTCTGAATACTCCTCTCTCAAG   660
V  A  G  S  K  L  V  L  R  C  E  T  S  S  E  Y  S  S  L  K
TTCAAGTGGTTCAAGAATGGGAGTGAATTAAGCCGAAAGAACAAACCACAAAACATCAAG   720
F  K  W  F  K  N  G  S  E  L  S  R  K  N  K  P  Q  N  I  K
ATACAGAAAAGGCCGGGGAAGTCAGAACTTCGCATTAGCAAAGCGTCACTGGCTGATTCT   780
I  Q  K  R  P  G  K  S  E  L  R  I  S  K  A  S  L  A  D  S
GGAGAATATATGTGCAAAGTGATCAGCAAACTAGGAAATGACAGTGCCTCTGCCAACATC   840
G  E  Y  M  C  K  V  I  S  K  L  G  N  D  S  A  S  A  N  I
ACCATTGTGGAGTCAAACGAGATCATCACTGGCATGCCAGCCTCAACTGAGACAGCGTAT   900
T  I  V  E  S  N  E  I  I  T  G  M  P  A  S  T  E  T  A  Y
GTGTCTTCAGAGTCTCCCATTAGAATATCAGTATCAACAGAAGGAACAAATACTTCTTCA   960
V  S  S  E  S  P  I  R  I  S  V  S  T  E  G  T  N  T  S  S
TCCACATCCACATCTACAGCTGGGACAAGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAA   1020
S  T  S  T  S  T  A  G  T  S  H  L  V  K  C  A  E  K  E  K
ACTTTCTGTGTGAATGGAGGCGAGTGCTTCATGGTGAAAGACCTTTCAAATCCCTCAAGA   1080
T  F  C  V  N  G  G  E  C  F  M  V  K  D  L  S  N  P  S  R
TACTTGTGCAAGTGCCCAAATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGGCC   1140
Y  L  C  K  C  P  N  E  F  T  G  D  R  C  Q  N  Y  V  M  A
AGCTTCTACAGTACGTCCACTCCCTTTCTGTCTCTGCCTGAATAGGCGCATGCTCAGTCG   1200
S  F  Y  S  T  S  T  P  F  L  S  L  P  E  *
GTGCCG[illegible]CATCT[illegible]GAGAT[illegible]GA[illegible]TAGAGCTAGATGCG[illegible]A   1260
[illegible]
```

BAD ORIGINAL

FIGURE 31 2/2

TGACTTCCTCTGTCCCGTGACTAGTCGGCTCTCAGCTACTCGTAGGTGCCGTAACCCTCCAG 1380
TGTTTCTGAAATTGATCTTGAATTACTGTGATACGACATGATAGTCCCTCTCACCCAGTG 1440
CAATGACAATAAAGCCCTTGAAAAGTCTCACTTTTATTGAGAAAATAAAAATCGTTCCAC 1500
GGGACAGTCCCCTCTTCTTTATAAAATGACCCTATCCTTGAAAAGGAGGTGTGTTAAGTG 1560
TAACCAGTACACACTTGAAATGATCGTAAGTTCGGTTCGGTTCAGAATGTGTTCTTTCTG 1620
ACAAATAAACAGAATAAAAAAAAAAAAAAAAAA 1653

BAD ORIGINAL

(SEQ ID NO: 145)

FIGURE 32

CGP28993 nucleotide sequence and deduced protein sequence

```
CATCAGGTGTGGGCCGCGAAAGCCGGCGGCCTGAAGAAGGACTCGCTGCTCACCGTGCGC   60
 H  Q  V  W  A  A  K  A  G  G  L  K  K  D  S  L  L  T  V  R
CTCGGCGCCGTCGGCCACCCCGCCCCTCCCTCCTGCGGCCGCCTCAAGGAGGACAGCAGG  120
 L  G  A  V  G  H  P  A  P  P  S  C  G  R  L  K  E  D  S  R
TACATCTTCTTCCATGAGCCCGAGGCCCACAGCAGCGGCGGCCCCGGCCGCCTTCCCAGC  180
 Y  I  F  F  H  E  P  E  A  H  S  S  G  G  P  G  R  L  P  S
CTCCTTCCCCCCTCTCGAGACGGGCCCGAACCTCAAGAAGGAGGTCAGCCCGGTGCTGTG  240
 L  L  P  P  S  R  D  G  P  E  P  Q  E  G  G  Q  P  G  A  V
CAACGGTGCGCCCTTCCTCCCCGGCTTGAGCAGATGAAGAGTCAGGAGTCTGTGGCAGGT  300
 Q  R  C  A  L  P  P  R  L  E  Q  M  K  S  Q  E  S  V  A  G
TCCAAACTAGTGCTTCGGTGCGAGACCAGTTCTGAATACTCCTCTCTCAAGTTCAAGGTG  360
 S  K  L  V  L  R  C  E  T  S  S  E  Y  S  S  L  K  F  K  V
TTCAAGAATGGCAGTGAGTTAAGCCGAAAGAACAAACCAGAAAACATCAAGATACAGAAA  420
 F  K  N  G  S  E  L  S  R  K  N  K  P  E  N  I  K  I  Q  K
AGGCCGGGGAAGTCAGAACTTCGCATTAGCAAAGCGTCACTGGCTGATTCTGGAGAATAT  480
 R  P  G  K  S  E  L  R  I  S  K  A  S  L  A  D  S  G  E  Y
ATGTGCAAAGTGATCAGCAAACTAGGAAATGACAGTGCCTCTGCCAACATCACCATTGTG  540
 M  C  K  V  I  S  K  L  G  N  D  S  A  S  A  N  I  T  I  V
GAGTCAAACGCCACATCCACATCTACAGCTGGGACAAGCCATCTTGTCAAGTGTGCAGAG  600
 E  S  N  A  T  S  T  S  T  A  G  T  S  H  L  V  K  C  A  E
AAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGCTTCATGGTGAAAGACCTTTCAAAT  660
 K  E  K  T  F  C  V  N  G  G  E  C  F  M  V  K  D  L  S  N
CCCTCAAGATACTTGTGCAAGTGCCAACCTGGATTCACTGGAGCGAGATGTACTGAGAAT  720
 P  S  R  Y  L  C  K  C  Q  P  G  F  T  G  A  R  C  T  E  N
GTGCCCATGAAAGTCCAAACCCAAGAAAAGTGCCCCAATGAGTTTACTGGTGATCGCTGC  780
 V  P  M  K  V  Q  T  Q  E  K  C  P  N  E  F  T  G  D  R  C
CAAAACTACGTAATGGCCAGCTTCTACAGTACGTCCACTCCCTTTCTGTCTCTGCCTGAA  840
 Q  N  Y  V  M  A  S  F  Y  S  T  S  T  P  F  L  S  L  P  E
TAGCGGCATCTCAGTCGGTGCCCGCTTTCTTGTTGCCGCATCTCCCTCAGATTCCTCCAG  900
 *
AGCTAGATGCCGTTTTACCAGGTCTAACATTGACTGCCTCTGGCCTGTCGGCATGAGAACATT  960
AACACAAGCCGATTGTATGACTTCCTCTGTCCGTGACTAGTGGGCTCTGAGCTACTCGTAG 1020
GTGCCGTAACGGCTCCAGTGTTTCTCAAATTCATCTTGAATTACTGTGATACCACATGATAG 1080
TCCCTCTCACCCAGTGCAATGACAATAAAGGCCTTGAAAGTCAAAAAAAAAAAAAAAAAA 1140
```

BAD ORIGINAL

(SEQ ID NO: 146)

FIGURE 33 1/2

GGF2BPP4 nucleotide sequence and deduced protein sequence

```
GAAGTCAGAACTTCGCATTAGCAAAGCCTCACTGGCTGATTCTGGAGAATATATGTGCAA   60
 K  S  E  L  R  I  S  K  A  S  L  A  D  S  G  E  Y  M  C  K
AGTGATCAGCAAACTAGGAAATGACAGTGCCTCTGCCAACATCACCATTGTGGAGTCAAA  120
 V  I  S  K  L  G  N  D  S  A  S  A  N  I  T  I  V  E  S  N
CGCCACATCCACATCTACAGCTGGGACAAGCCATCTTGTCAAGTGTGCAGAGAAGGAGAA  180
 A  T  S  T  S  T  A  G  T  S  H  L  V  K  C  A  E  K  E  K
AACTTTCTGTGTGAATGGAGGCGACTGCTTCATGGTGAAAGACCTTTCAAATCCCTCAAG  240
 T  F  C  V  N  G  G  D  C  F  M  V  K  D  L  S  N  P  S  R
ATACTTGTGCAAGTGCCAACCTGGATTCACTGGAGCCAGATGTACTGAGAATGTGCCCAT  300
 Y  L  C  K  C  Q  P  G  F  T  G  A  R  C  T  E  N  V  P  M
GAAAGTCCAAACCCAAGAAAAAGCCGAGGAGCTCTACCAGAAGAGAGTGCTCACCATTAC  360
 K  V  Q  T  Q  E  K  A  E  E  L  Y  Q  K  R  V  L  T  I  T
CGGCATTTGCATCGCGCTGCTCGTGGTTGGCATCATGTGTGTGGTGGTCTACTGCAAAAC  420
 G  I  C  I  A  L  L  V  V  G  I  M  C  V  V  V  Y  C  K  T
CAAGAAACAACGGAAAAAGCTTCATGACCGGCTTCGGCAGAGCCTTCGGTCTGAAAGAAA  480
 K  K  Q  R  K  K  L  H  D  R  L  R  Q  S  L  R  S  E  R  N
CACCATGATGAACGTAGCCAACGGGCCCCACCACCCCAATCCGCCCCCCGAGAACGTGCA  540
 T  M  M  N  V  A  N  G  P  H  H  P  N  P  P  P  E  N  V  Q
GCTGGTGAATCAATACGTATCTAAAAATGTCATCTCTAGCGAGCATATTGTTGAGAGAGA  600
 L  V  N  Q  Y  V  S  K  N  V  I  S  S  E  H  I  V  E  R  E
GGCCGAGAGCTCTTTTTCCACCAGTCACTACACTTCGACAGCTCATCATTCCACTACTGT  660
 A  E  S  S  F  S  T  S  H  Y  T  S  T  A  H  H  S  T  T  V
CACTCAGACTCCCAGTCACAGCTGGAGCAATGGACACACTGAAAGCATCATTTCCGAAAG  720
 T  Q  T  P  S  H  S  W  S  N  G  H  T  E  S  I  I  S  E  S
CCACTCTGTCATCGTGATGTCATCCGTAGAAAACAGTAGGCACAGCAGCCCCACTGGGGG  780
 H  S  V  I  V  M  S  S  V  E  N  S  R  H  S  S  P  T  G  G
CCCCAGAGGACGTCTCAATGGCTTGGGAGGCCCTCGTGAATGTAACAGCTTCCTCAGGCA  840
 P  R  G  R  L  N  G  L  G  G  P  R  E  C  N  S  F  L  R  H
TGCCAGAGAAACCCCTGACTCCTACCGAGACTCTCCTCATAGTGAAAGACATAACCTTAT  900
 A  R  E  T  P  D  S  Y  R  D  S  P  H  S  E  R  H  N  L  I
AGCTGAGCTAAGGAGAAACAAGGCCCACAGATCCAAATGCATGCAGATCCAGCTTTCCGC  960
 A  E  L  R  R  N  K  A  H  R  S  K  C  M  Q  I  Q  L  S  A
AACTCATCTTAGAGCTTCTTCCATTCCCCATTGGGCTTCATTCTCTAAGACCCCTTGGCC 1020
 T  H  L  R  A  S  S  I  P  H  W  A  S  F  S  K  T  P  W  P
TTCAGCAAGGTATCTATCAGCCAATCACCACCCCCGGCTCGTATGTCACCTCTAGATTTCCA 1080
 L  G  R  Y  V  S  A  N  T  T  P  A  R  N  S  P  V  D  F  H
CACC[illegible]AAG[illegible]AAG[illegible]CAT[illegible]TC[illegible]AAATGTC[illegible]CC[illegible]C[illegible]AGCAC[illegible]AC [illegible]
 [illegible]  P  S  S  [illegible]  Y  S  P  P  S  E  [illegible]  S  P  P  [illegible]  S  S  [illegible]  [illegible]
```

BAD ORIGINAL

FIGURE 33 2/2

GGTCTCCCATCCCTCCCATGCGGTCAGTCCCTTCGTGGAAGAGGAGAGACCCCTGCTCCT 1200
V S H P S H A V S P F V E E E R P L L L

TGTGACGCCCCCACGGCTGCGGGAGAAGTATGACCACCACGCCCAGCAATTCCACTCGTT 1260
V T P P R L R E K Y D H H A Q Q F H S F

CCACTGCCACCCCGCGCATGAGAGCCACAGCCTGCCCCCCAGCCCCCTGAGGATAGTGGA 1320
H C H P A H E S H S L P P S P L R I V E

GGATGAGGAATATGAAACGACCCAGGAGTACGAACCAGCTCAAGAGCCCGTTAAGGAACT 1380
D E E Y E T T Q E Y E P A Q E P V K E L

CACCAACAGCAGCCGCCGCGCCAAAAGAACCAAGCCCAATGGTCACATTGCCCACAGGTT 1440
T N S S R R A K R T K P N G H I A H R L

GGAAATGGACAACAACACAGGCGCTGACAGCAGTAACTCAGAGAGCGAAACAGAGGATGA 1500
E M D N N T G A D S S N S E S E T E D E

AAGAGTAGGAGAAGATACGCCTTTCCTGGCCATACAGAACCCCCTGGCAGCCAGTCTCGA 1560
R V G E D T P F L A I Q N P L A A S L E

GGCGGCCCCCGCCCCTCGCCTGGTCGACAGCAGGACTAACCCAACAGGCGGCTTCTCTCC 1620
A A P A P R L V D S R T N P T G G F S P

GCAGGAAGAATTGCAGGCCAGGCTCTCCGGTGTAATCGCTAACCAAGACCCTATCGCTGT 1680
Q E E L Q A R L S G V I A N Q D P I A V

CTAAAACCCGAAATACACCCATAGATTCACCTGTAAAACTTTATTTTATATAATAAAGTAT 1740
*

TCCACCTTAAATTAAACAAAAAAA 1764

BAD ORIGINAL

FIGURE 34

```
                    •        •        •                  •  •           •
GGF2bpp5 KCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFY[1]
GGF2bpp4 KCAEKEKTFCVNGGDCFMVKDLSNPSRYLCKCQPGFTGARCTENVPMKVQ[2]
hEGF     ECLRKYKDFCIH-GECKYVKELRAPS— CKCQQEYFGERCGEKSNKTHS[3]
```

[1](SEQ ID NO: 147)
[2](SEQ ID NO: 148)
[3](SEQ ID NO: 149)

FIGURE 35

200 kDa tyrosine phosphorylation compared with mitogenic activity

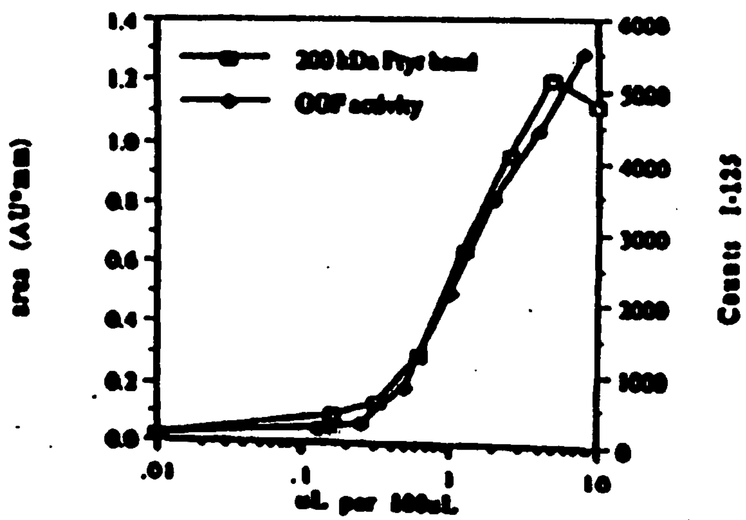


FIGURE 36 1/2

GGF/HEREGULIN SPLICING VARIANTS

F-B-A'

F-B-A-C-C/D-D
F-B-A-C-C/D-H
F-B-A-C-C/D-H-L
F-B-A-C-C/D-H-K-L
F-B-A-C-C/D-D'-H
F-B-A-C-C/D-D'-H-L
F-B-A-C-C/D-D'-H-K-L
F-B-A-C-C/D'-D
F-B-A-C-C/D'-H
F-B-A-C-C/D'-H-L
F-B-A-C-C/D'-H-K-L
F-B-A-C-C/D'-D'-H
F-B-A-C-C/D'-D'-H-L
F-B-A-C-C/D'-D'-H-K-L
F-B-A-C-C/D-C/D'-D
F-B-A-C-C/D-C/D'-H
F-B-A-C-C/D-C/D'-H-L
F-B-A-C-C/D-C/D'-H-K-L
F-B-A-C-C/D-C/D'-D'-H
F-B-A-C-C/D-C/D'-D'-H-L
F-B-A-C-C/D-C/D'-D'-H-K-L

F-E-B-A'

F-E-B-A-C-C/D-D
F-E-B-A-C-C/D-H
F-E-B-A-C-C/D-H-L
F-E-B-A-C-C/D-H-K-L
F-E-B-A-C-C/D-D'-H
F-E-B-A-C-C/D-D'-H-L
F-E-B-A-C-C/D-D'-H-K-L
F-E-B-A-C-C/D'-D
F-E-B-A-C-C/D'-H
F-E-B-A-C-C/D'-H-L
F-E-B-A-C-C/D'-H-K-L
F-E-B-A-C-C/D'-D'-H
F-E-B-A-C-C/D'-D'-H-L
F-E-B-A-C-C/D'-D'-H-K-L
F-E-B-A-C-C/D-C/D'-D
F-E-B-A-C-C/D-C/D'-H
F-E-B-A-C-C/D-C/D'-H-L
F-E-B-A-C-C/D-C/D'-H-K-L
F-E-B-A-C-C/D-C/D'-D'-H
F-E-B-A-C-C/D-C/D'-D'-H-L
F-E-B-A-C-C/D-C/D'-D'-H-K-L

F-B-A-G-C-C/D-D
F-B-A-G-C-C/D-H
F-B-A-G-C-C/D-H-L
F-B-A-G-C-C/D-H-K-L
F-B-A-G-C-C/D-D'-H
F-B-A-G-C-C/D-D'-H-L
F-B-A-G-C-C/D-D'-H-K-L
F-B-A-G-C-C/D'-D
F-B-A-G-C-C/D'-H
F-B-A-G-C-C/D'-H-L
F-B-A-G-C-C/D'-H-K-L
F-B-A-G-C-C/D'-D'-H
F-B-A-G-C-C/D'-D'-H-L
F-B-A-G-C-C/D'-D'-H-K-L
F-B-A-G-C-C/D-C/D'-D
F-B-A-G-C-C/D-C/D'-H
F-B-A-G-C-C/D-C/D'-H-L
F-B-A-G-C-C/D-C/D'-H-K-L
F-B-A-G-C-C/D-C/D'-D'-H
F-B-A-G-C-C/D-C/D'-D'-H-L
F-B-A-G-C-C/D-C/D'-D'-H-K-L

F-E-B-A-G-C-C/D-D
F-E-B-A-G-C-C/D-H
F-E-B-A-G-C-C/D-H-L
F-E-B-A-G-C-C/D-H-K-L
F-E-B-A-G-C-C/D-D'-H
F-E-B-A-G-C-C/D-D'-H-L
F-E-B-A-G-C-C/D-D'-H-K-L
F-E-B-A-G-C-C/D'-D
F-E-B-A-G-C-C/D'-H
F-E-B-A-G-C-C/D'-H-L
F-E-B-A-G-C-C/D'-H-K-L
F-E-B-A-G-C-C/D'-D'-H
F-E-B-A-G-C-C/D'-D'-H-L
F-E-B-A-G-C-C/D'-D'-H-K-L
F-E-B-A-G-C-C/D-C/D'-D
F-E-B-A-G-C-C/D-C/D'-H
F-E-B-A-G-C-C/D-C/D'-H-L
F-E-B-A-G-C-C/D-C/D'-H-K-L
F-E-B-A-G-C-C/D-C/D'-D'-H
F-E-B-A-G-C-C/D-C/D'-D'-H-L
F-E-B-A-G-C-C/D-C/D'-D'-H-K-L

FIGURE 36 2/2

GGF/HEREGULIN SPLICING VARIANTS CONTINUED

E-B-A'

E-B-A-C-C/D-D
E-B-A-C-C/D-H
E-B-A-C-C/D-K-L
E-B-A-C-C/D-H-K-L
E-B-A-C-C/D-D'-H
E-B-A-C-C/D-D'-K-L
E-B-A-C-C/D-D'-H-K-L
E-B-A-C-C/D'-D
E-B-A-C-C/D'-H
E-B-A-C-C/D'-K-L
E-B-A-C-C/D'-H-K-L
E-B-A-C-C/D'-D'-H
E-B-A-C-C/D'-D'-K-L
E-B-A-C-C/D'-D'-H-K-L
E-B-A-C-C/D-C/D'-D
E-B-A-C-C/D-C/D'-H
E-B-A-C-C/D-C/D'-K-L
E-B-A-C-C/D-C/D'-H-K-L
E-B-A-C-C/D-C/D'-D'-H
E-B-A-C-C/D-C/D'-D'-K-L
E-B-A-C-C/D-C/D'-D'-H-K-L

E-B-A-G-C-C/D-D
E-B-A-G-C-C/D-H
E-B-A-G-C-C/D-K-L
E-B-A-G-C-C/D-H-K-L
E-B-A-G-C-C/D-D'-H
E-B-A-G-C-C/D-D'-K-L
E-B-A-G-C-C/D-D'-H-K-L
E-B-A-G-C-C/D'-D
E-B-A-G-C-C/D'-H
E-B-A-G-C-C/D'-K-L
E-B-A-G-C-C/D'-H-K-L
E-B-A-G-C-C/D'-D'-H
E-B-A-G-C-C/D'-D'-K-L
E-B-A-G-C-C/D'-D'-H-K-L
E-B-A-G-C-C/D-C/D'-D
E-B-A-G-C-C/D-C/D'-H
E-B-A-G-C-C/D-C/D'-K-L
E-B-A-G-C-C/D-C/D'-H-K-L
E-B-A-G-C-C/D-C/D'-D'-H
E-B-A-G-C-C/D-C/D'-D'-K-L
E-B-A-G-C-C/D-C/D'-D'-H-K-L

EGFL1


```
AGCCATCTTGTCAAGTGTGCCGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGC
S  H  L  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C

TTCATGGTGAAAGACCTTTCAAATCCCTCAAGATACTTGTGCAAGTGCCCAAATGAGTTT
F  M  V  K  D  L  S  N  P  S  R  Y  L  C  K  C  P  N  E  F

ACTGGTGATCGCTGCCAAAACTACGTAATGGCCAGCTTCTACAGTACGTCCACTCCCTTT
T  G  D  R  C  Q  N  Y  V  M  A  S  F  Y  S  T  S  T  P  F

CTGTCTCTGCCTGAATAG
L  S  L  P  E  *
```

(SEQ ID NO: 150)

FIGURE 37

BAD ORIGINAL

EGFL2

AGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGC
S H L V K C A E K E K T F C V N G G E C

TTCATGGTGAAAGACCTTTCAAATCCCTCAAGATACTTGTGCAAGTGCCAACCTGGATTC
F M V K D L S N P S R Y L C K C Q P G F

ACTGGAGCCAGATGTACTGAGAATGTGCCCATGAAAGTCCAAACCCAAGAAAAAGCGGAG
T G A R C T E N V P M K V Q T Q E K A E

GAGCTCTACTAA
E L Y *

(SEQ ID NO: 15/)

FIGURE 38

BAD ORIGINAL

EGFL3


```
AGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGC
 S  H  L  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C
TTCATGGTGAAAGACCTTTCAAATCCCTCAAGATACTTGTGCAAGTGCCCAAATGAGTTT
 F  M  V  K  D  L  S  N  P  S  R  Y  L  C  K  C  P  N  E  F
ACTGGTGATCGCTGCCAAAACTACGTAATGGCCAGCTTCTACAAGGCGGAGGAGCTCTAC
 T  G  D  R  C  Q  N  Y  V  M  A  S  F  Y  K  A  E  E  L  Y
TAA
 *
```

(SEQ ID NO: 152)

FIGURE 39

BAD ORIGINAL

EGFLA

```
AGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGC
S  H  L  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C

TTCATGGTCAAAGACCTTTCAAATCCCTCAAGATACTTGTGCAAGTGCCCAAATGAGTTT
F  M  V  K  D  L  S  N  P  S  R  Y  L  C  K  C  P  N  E  F

ACTGGTGATCGCTGCCAAAACTACGTAATGGCCAGCTTCTACAAGCATCTTGGGATTGAA
T  G  D  R  C  Q  N  Y  V  M  A  S  F  Y  K  H  L  G  I  E

TTTATGGAGAAAGCGGAGGAGCTCTACTAA
F  M  E  K  A  E  E  L  Y  *
```

(SEQ ID NO: 153)

FIGURE 40

BAD ORIGINAL

EGFLS


```
AGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGC
 S  H  L  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C
TTCATGGTGAAAGACCTTTCAAATCCCTCAAGATACTTGTGCAAGTGCCAACCTGGATTC
 F  M  V  K  D  L  S  N  P  S  R  Y  L  C  K  C  Q  P  G  F
ACTGGAGCCAGATGTACTGAGAATGTGCCCATGAAAGTCCAAACCCAAGAAAAGTGCCCA
 T  G  A  R  C  T  E  N  V  P  M  K  V  Q  T  Q  E  K  C  P
AATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGGCCAGCTTCTACAGTACGTCC
 N  E  F  T  G  D  R  C  Q  N  Y  V  M  A  S  F  Y  S  T  S
ACTCCCTTTCTGTCTCTGCCTGAATAG
 T  P  F  L  S  L  P  E  *
```

(SEQ ID NO: 154)

FIGURE 41

BAD ORIGINAL

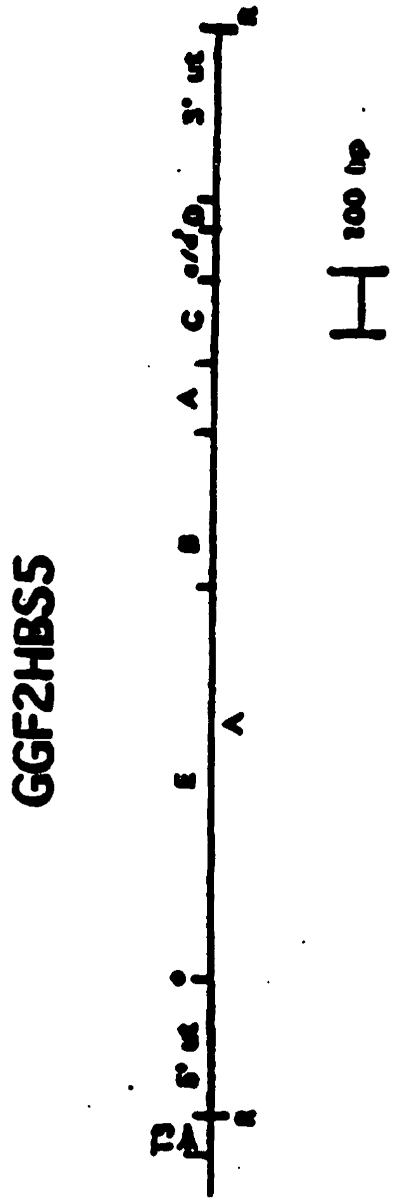
EGFL6


```
AGCCATCTTGTCAAGTGTGCAGAGAAGGAGAAAACTTTCTGTGTGAATGGAGGCGAGTGC
 S  H  L  V  K  C  A  E  K  E  K  T  F  C  V  N  G  G  E  C

TTCATGGTGAAAGACCTTTCAAATCCCTCAAGATACTTGTGCAAGTGCCAACCTGGATTC
 F  M  V  K  D  L  S  N  P  S  R  Y  L  C  K  C  Q  P  G  F

ACTGGAGCGAGATGTACTGAGAATGTGCCCATGAAAGTCCAAACCCAAGAAAAGTGCCCA
 T  G  A  R  C  T  E  N  V  P  M  K  V  Q  T  Q  E  K  C  P

AATGAGTTTACTGGTGATCGCTGCCAAAACTACGTAATGGCCAGCTTCTACAAAGCCGAG
 N  E  F  T  G  D  R  C  Q  N  Y  V  M  A  S  F  Y  K  A  E

GAGCTCTACTAA
 E  L  Y  *
```

(SEQ ID NO: 155)

FIGURE 42

FIGURE 43



Nucleotide sequence and deduced amino acid sequence of [illegible]

[illegible] 60

[illegible] 120

[illegible] 180

[illegible] 240

[illegible] 300

[illegible] 360

[illegible] 420

[illegible] 480

[illegible] 540

[illegible] 600

[illegible] 660

[illegible] 720

(SEQ ID NO: 21)

FIGURE 44 1/3

BAD ORIGINAL

[illegible] 780

[illegible] 840

[illegible] 900

[illegible] 960

[illegible] 1020

[illegible] 1080

[illegible] 1140

[illegible] 1200

[illegible] 1260

[illegible] 1320

FIGURE 44 2/3

BAD ORIGINAL

[illegible] 1380
[illegible]

[illegible] 1440
[illegible]

[illegible] 1500
[illegible]

[illegible] 1560
[illegible]

[illegible] 1620

[illegible] 1680

[illegible] 1740

[illegible] 1800

[illegible] 1860

[illegible] 1920

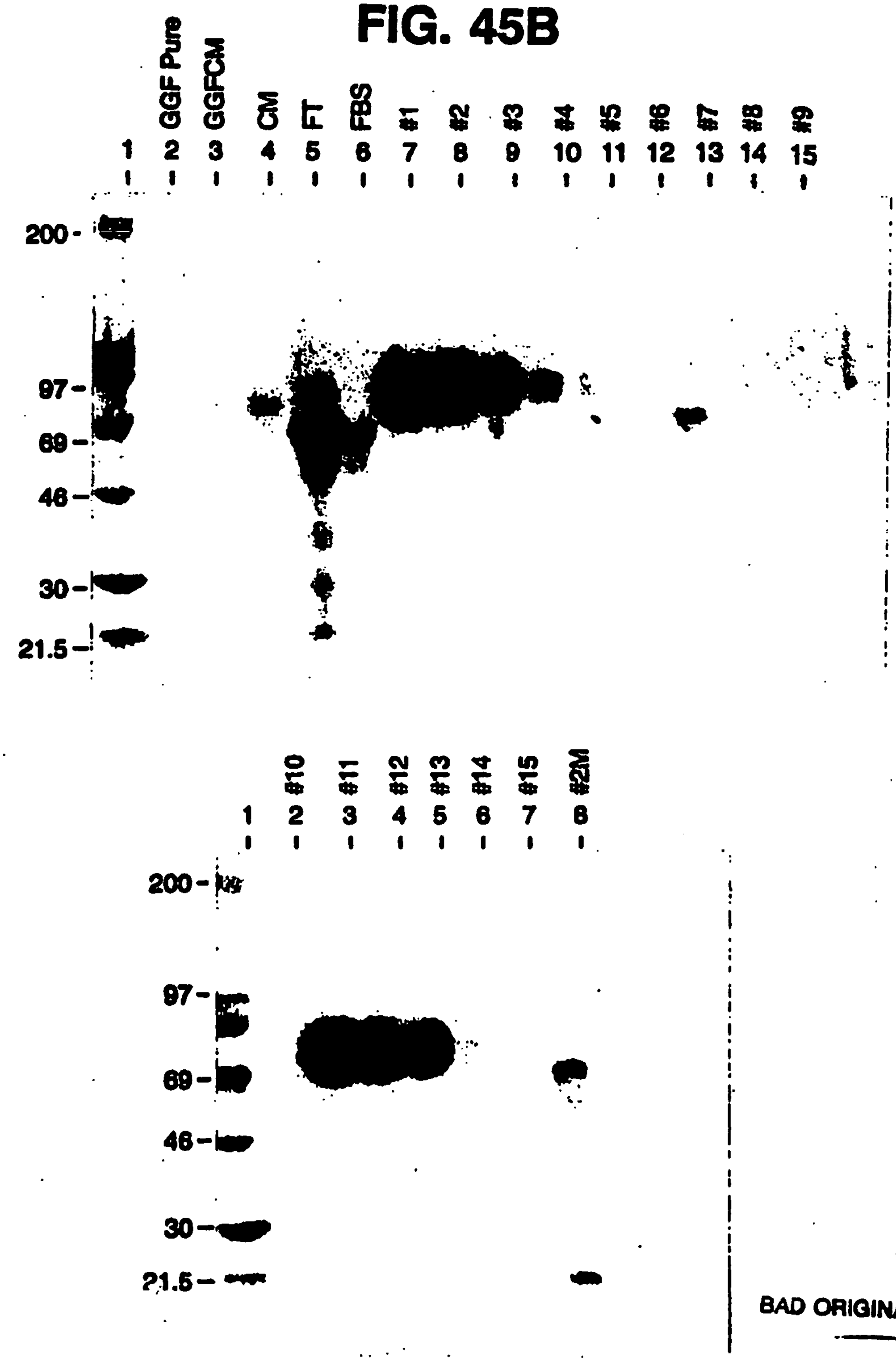
[illegible] 1980

[illegible] 2003

FIGURE 44 3/3

BAD ORIGINAL

FIG. 45B



BAD ORIGINAL

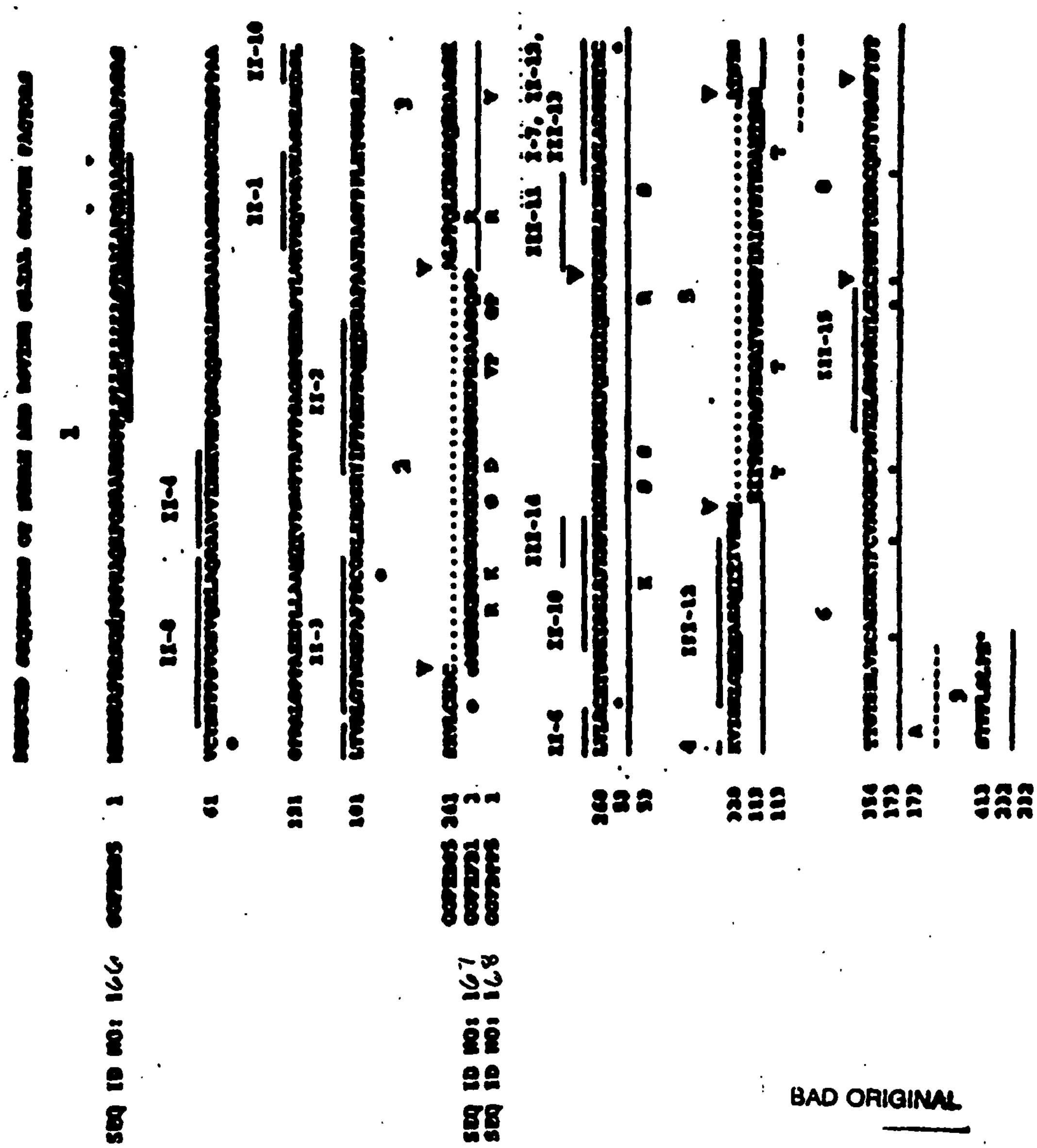


FIGURE 46

BAD ORIGINAL

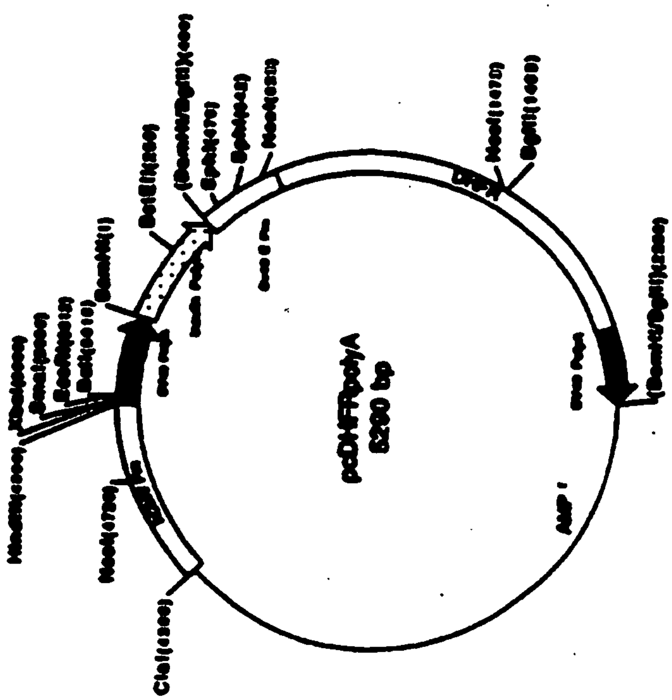


FIGURE 47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05083

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/10, 37/36
US CL :514/8, 12; 530/399
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : N/A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	N/A	

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 22 SEPTEMBER 1994	Date of mailing of the international search report 30 SEP 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SHELLY GUEST CERMAK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNA IAL SEARCH REPORT

national application No.
PCT/US94/05083

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-91
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

because applicants have failed to submit a searchable computer Sequence Listing, and each of the claims encompasses DNA or amino acid sequences.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.